Supplementary Information
for

A biohybrid valveless pump-bot powered by engineered skeletal muscle

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Supplementary Information Text

Reynolds number and Womersley number.

The Reynolds number in a steady flow is defined as,

$$R_e = \frac{\rho \bar{u} d}{\mu_f} \quad [1]$$

where ρ is the density of fluid, $\bar{u}$ is a characteristic velocity, d is a characteristic length, and μ_f is the dynamic viscosity of fluid. For our pump-bot systems, the fluid is the cell culture medium. The density ρ is obtained by the dividing the mass by the volume, which is measured to be 0.987-1.008 g/ml at 37°C in the experiment. $\bar{u}$ is defined as the mean axial velocity which is the half of maximum axial velocity, V_{max}, and d as the inner diameter of hydrogel tube. The dynamic viscosity for cell culture medium at 37°C is $\mu_f = 0.82 mPa \cdot s$ (1).

For our pump-bots, the flow also fluctuates periodically with muscle actuation cycles. The Womersley number is defined as the ratio of the inertial forces to the viscous forces for the pulsatile flows and can be expressed as,

$$\alpha = \frac{d}{2}\sqrt{\frac{\rho \omega}{\mu_f}} \quad [2]$$

where $\omega = 2\pi f$ is a characteristic frequency in radians per second. For our pump-bots, f is the frequency of muscle spontaneous twitching or muscle electrical stimulations. Reynolds number and Womersley number for all cases are given in Table S1, where the maximum velocity, V_{max}, at the mid region of the tube is used to calculate the Reynolds number.

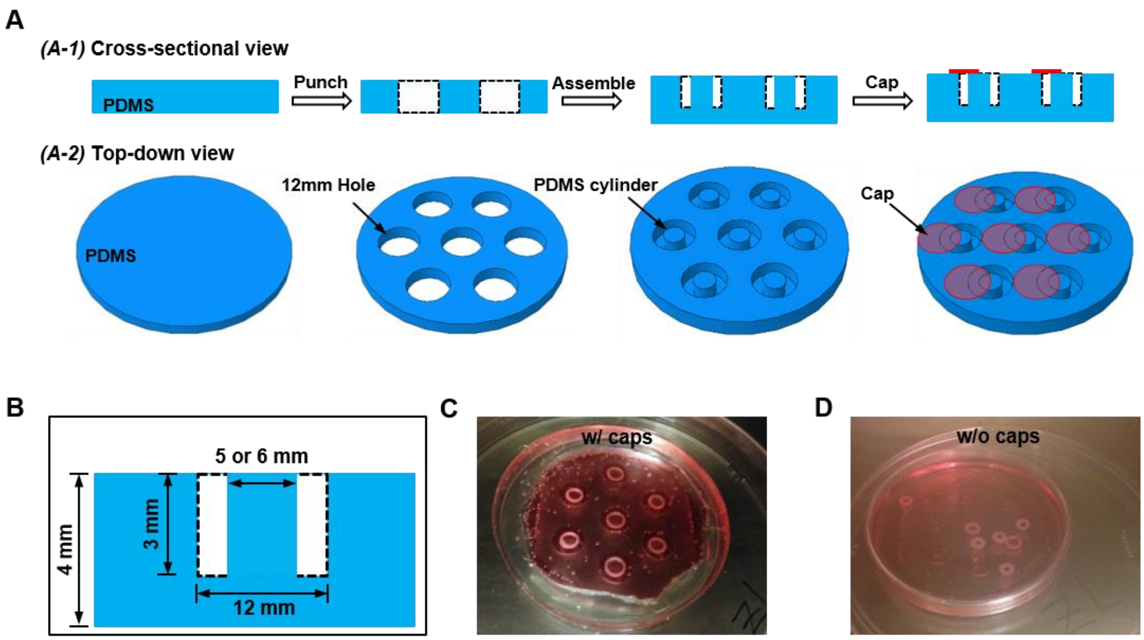


Fig. S1. The cross-sectional view *(A-1)* and top-down view *(A-2)* of the fabrication process for making PDMS muscle ring mold. *(B)* The dimensions of one unit muscle ring mold. *(C)* Engineered muscle rings fabricated by using capped muscle ring mold. *(D)* Muscle rings escape from the mold without the caps.

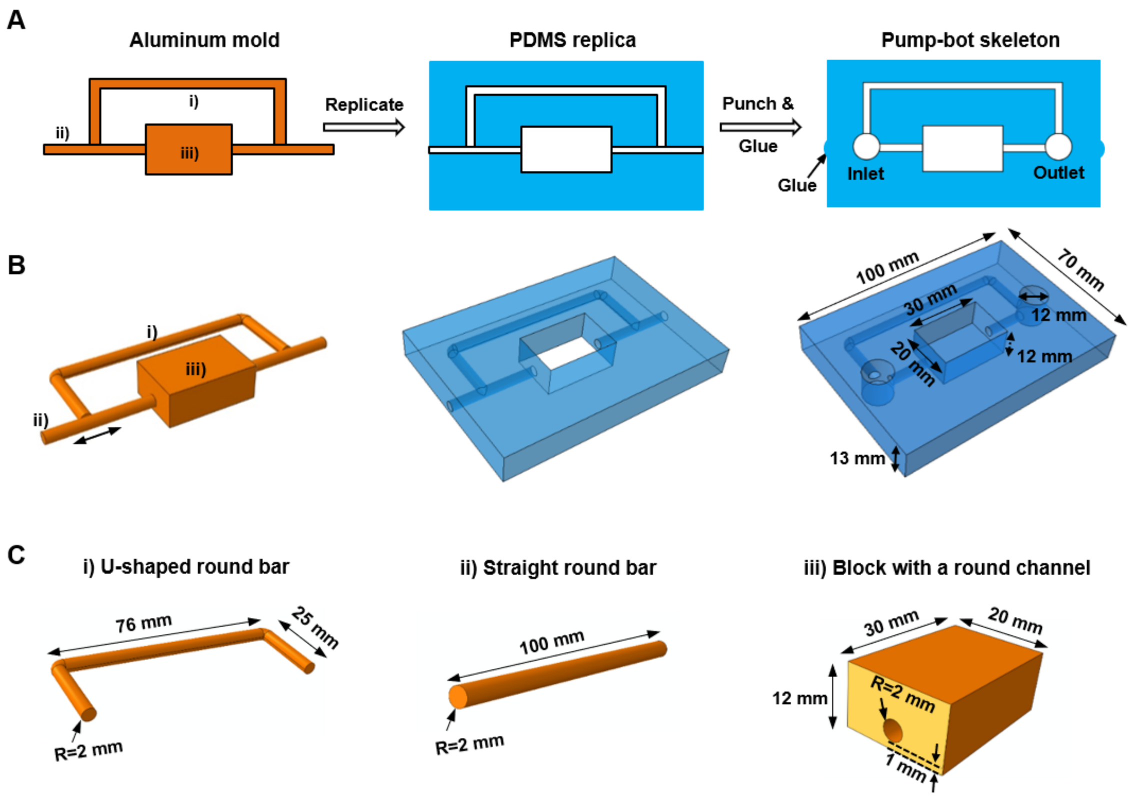


Fig. S2. Schematics of the fabrication process for making pump-bot skeleton with dimensions shown in two dimensional *(A)* and three dimensional *(B)* forms respectively. *(C)* The dimensions of aluminum mold components including U-shaped round bar (i), straight round bar (ii) and one block with a round channel inside (iii).

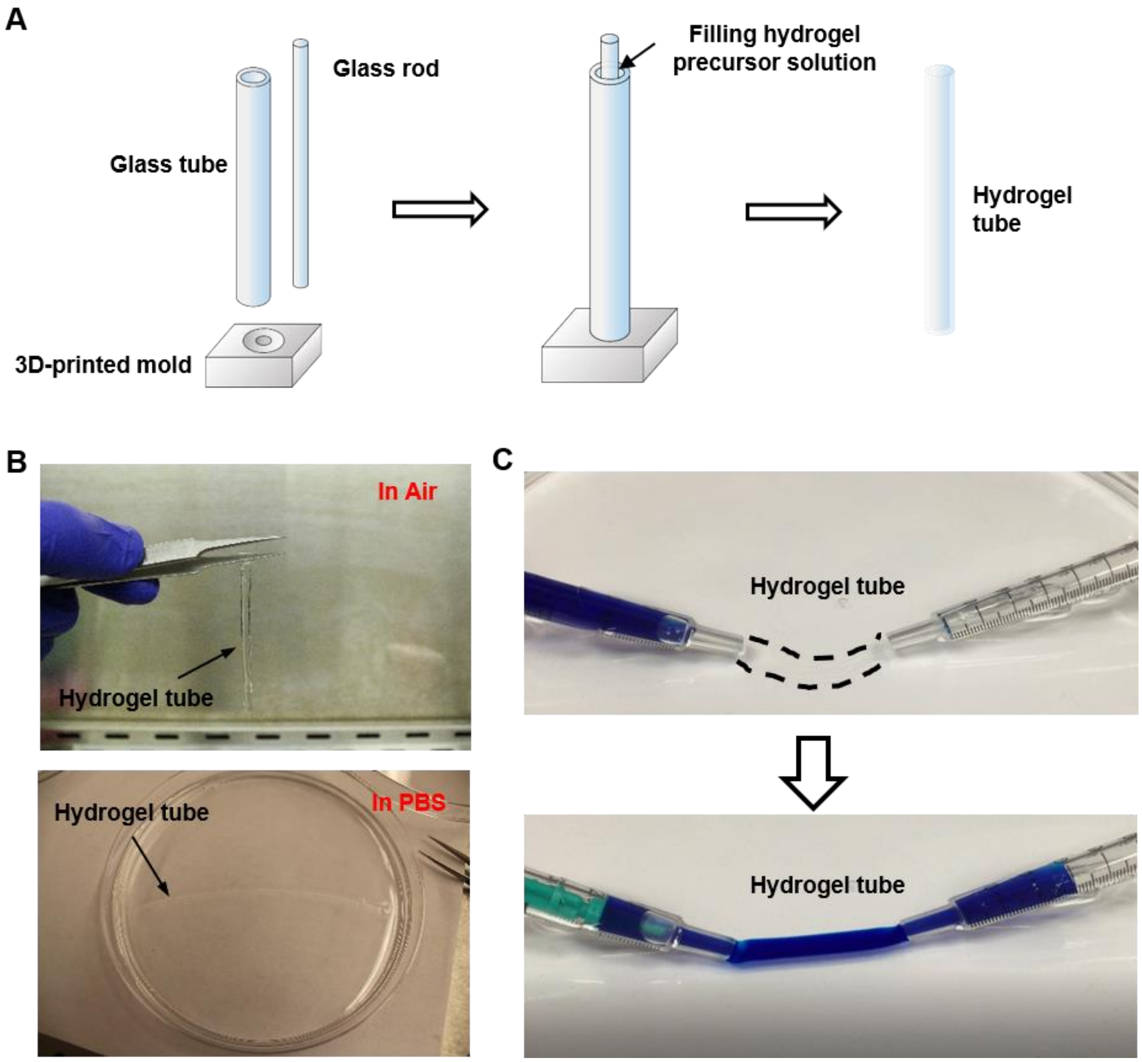


Fig. S3. *(A)* Schematic illustration of the fabrication process for preparing hydrogel tube. *(B)* The hydrogel tube collapses in air due to the dehydration but it recovers to the initial round shape in the liquid environment (phosphate buffered saline, PBS). *(C)* Leakage test of the hydrogel tube.

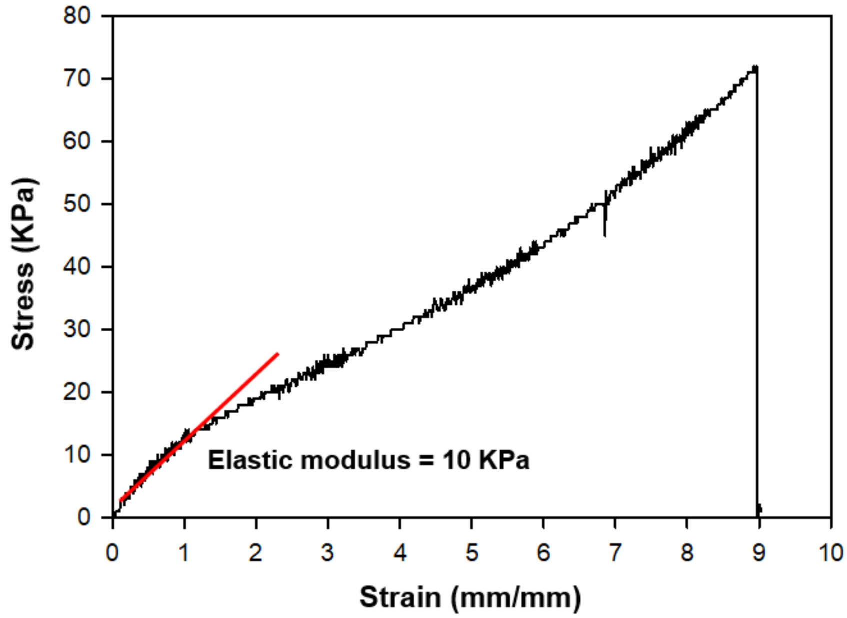


Fig. S4. Mechanical properties for the hydrogel tube. Stress-strain curve shows that the elastic Young's modulus of the hydrogel tubes is around 10 KPa and these tough hydrogel tubes can be stretched up to 9 times before the fracture.

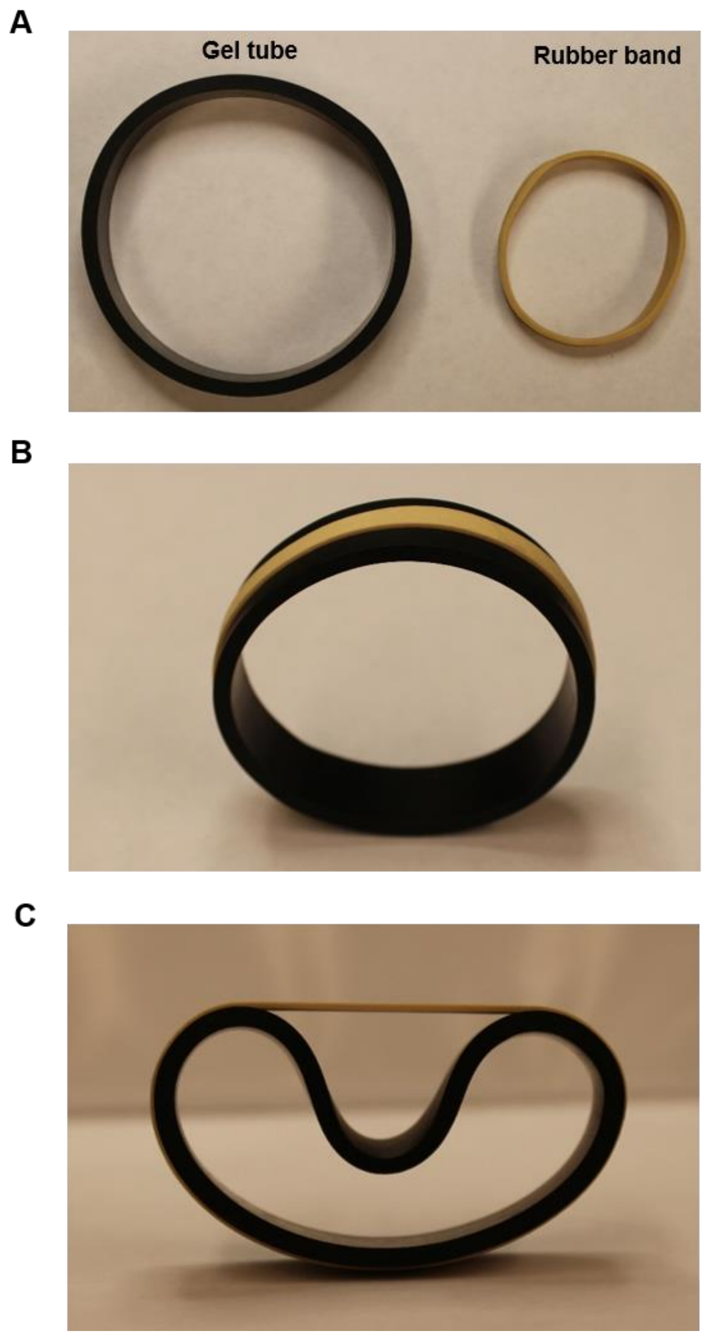


Fig. S5. A simple demonstration of buckling of a soft tube under static muscle contraction. *(A)* Image of the tube and rubber band. *(B)* No buckling occurred under the tension of rubber band. *(C)* Buckled tube due to rubber band tension.

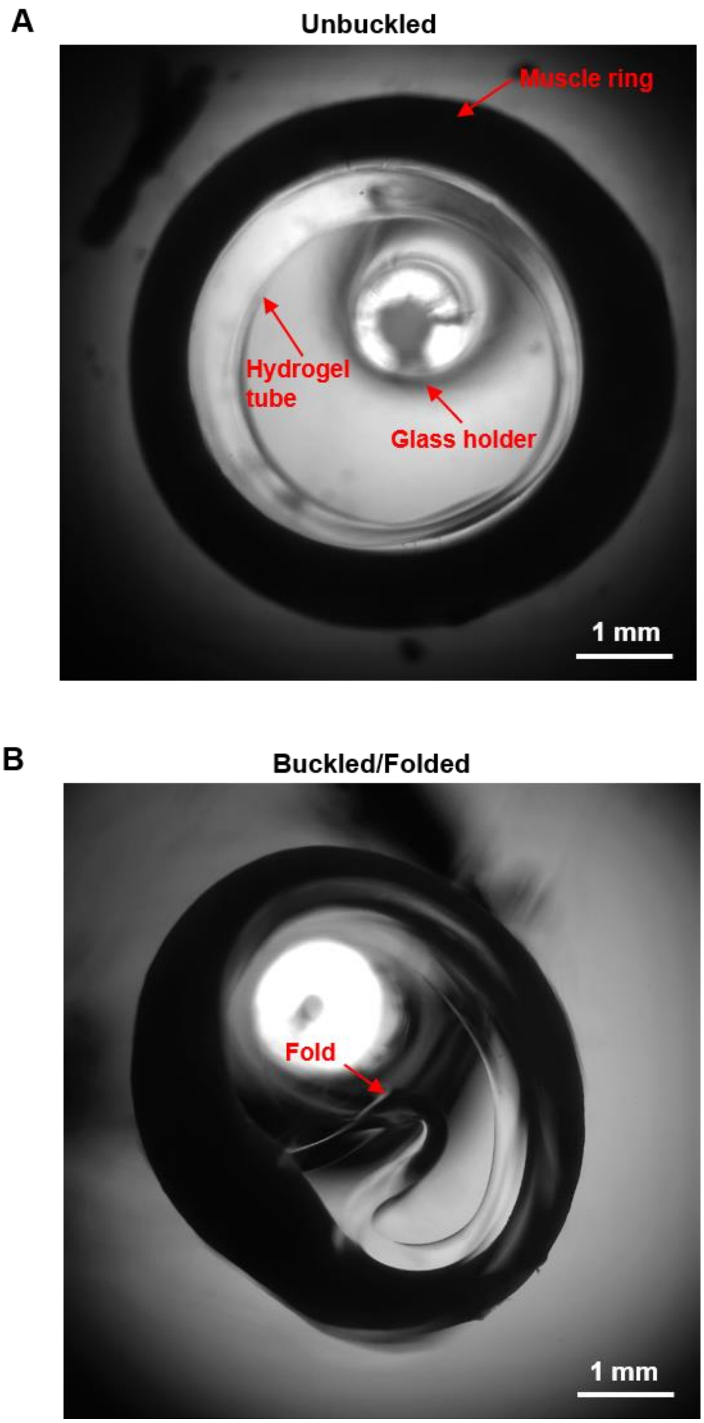
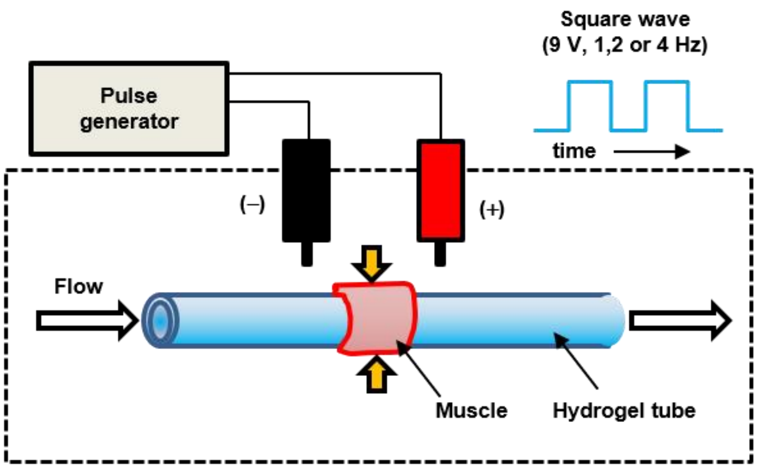


Fig. S6. The cross-sectional view of hydrogel tube under static muscle contraction. Phase contrast image of a muscle ring wrapping around the *(A)* unbuckled and *(B)* buckled hydrogel tube.

A



Electrically stimulate muscle tissues to induce flow

B

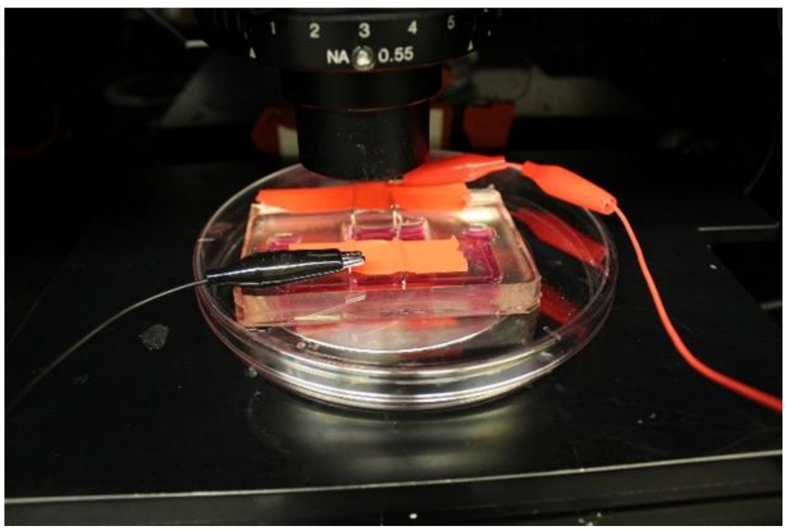


Fig. S7. *(A) S*chematic illustration of electrical stimulation setup. *(B)* Photography of the electrical stimulation and imaging setup.

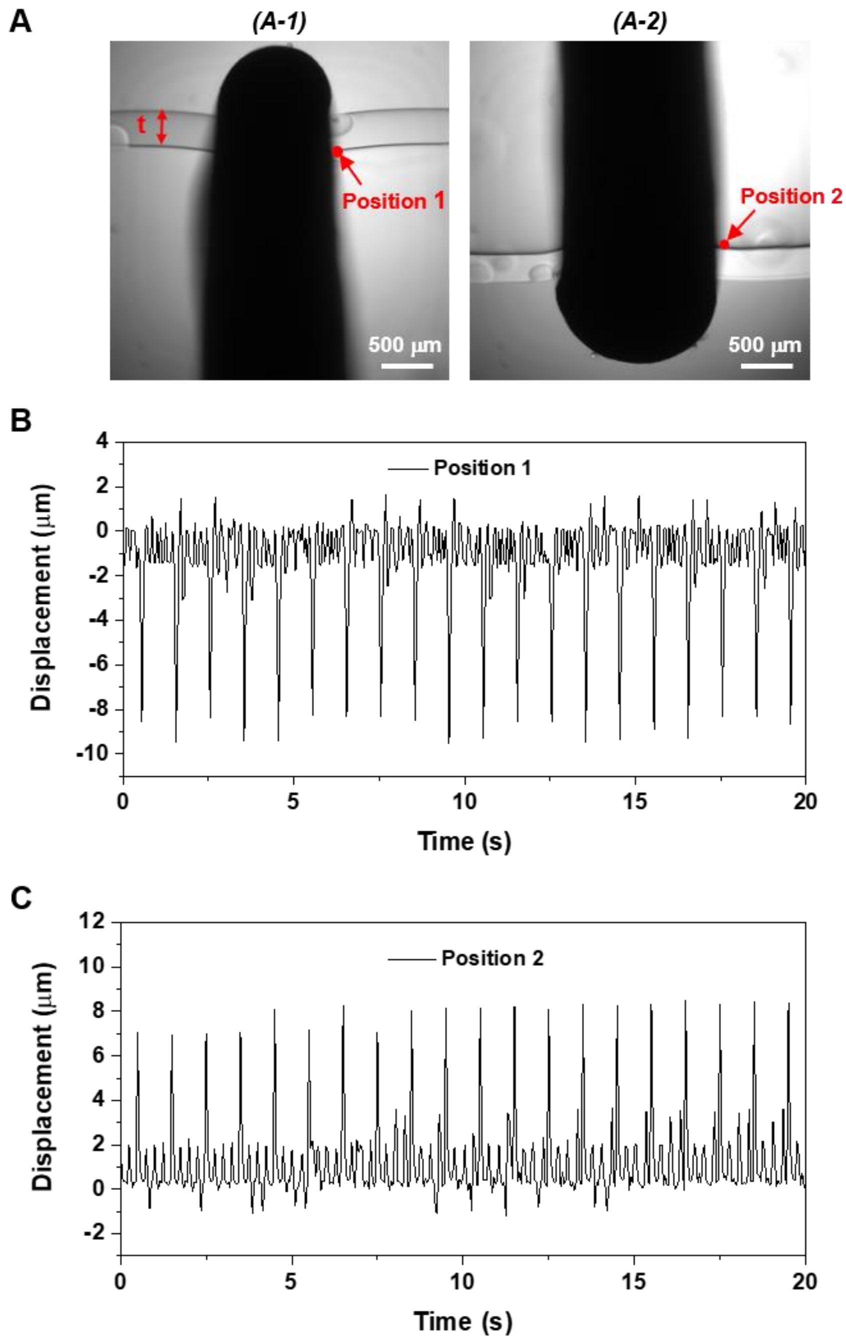


Fig. S8. *(A)* Phase contrast image of a muscle ring wrapping around the hydrogel tube. Time varying deformation of hydrogel tube at position1 *(B)* and position2 *(C)* induced by muscle ring subjected to 1Hz electrical stimulation.

Table S1. Output parameters for sample pump-bots.

	Voltage (V)	f (Hz)	V_{max} (μm/s)	Q (μl/min)	α	Re
Pump-bot 1 (self-twitching muscle)	NA	1.33	39.9	11.62	5.87	0.08
Pump-bot 2 (electrically stimulated muscle)	9	1	38.5	12.60	4.90	0.09
	9	2	27.0	8.28	6.93	0.05
	9	4	73.2	22.68	9.80	0.15
Pump-bot 3 (electrically stimulated muscle, tube buckled/folded)	4.5	1	13.84	3.1	4.54	0.02
	4.5	2	20.27	4.13	6.03	0.03
	4.5	4	31.89	8.91	9.36	0.07
	9	1	34.4	5.36	3.57	0.05
	9	2	59.5	11.44	5.48	0.11
	9	4	71.6	13.69	7.70	0.13

Movie S1. Spontaneous twitching of a muscle on top of the hydrogel tube.

Movie S2. The motion of florescent beads indicating the unidirectional flow induced by muscle spontaneous twitching.

Movie S3. A trajectory of fluorescent bead detected at the radial distance of 0.65 mm from tube center, with the average velocity of 31.7 μm/s.

Movie S4. No flow is observed in the experiment when spontaneous muscle twitching diminishes.

Movie S5. The change of cross-section of an un-buckled hydrogel tube under 4 Hz electrical stimulation.

Movie S6. Periodic muscle contraction on top of an unbuckled hydrogel tube under 1 Hz electrical stimulation.

Movie S7. The change of cross-section of a buckled hydrogel tube induced by muscle spontaneous twitching.

Movie S8. Cross sectional view of buckled hydrogel tube under 4 Hz electrical stimulation with the voltage of 9 V.

Movie S9. The trajectory of a fluorescent bead under 4 Hz electrical stimulation with the voltage of 9 V showing that the flow is fluctuating with the cyclic external stimulation, but the overall movement is unidirectional.

References

1. Williams BJ, Anand S V., Rajagopalan J, Saif MTA (2014) A self-propelled biohybrid swimmer at low Reynolds number. *Nat Commun* 5:3081.