Supplementary Information for:

Development of Miniaturized Walking Biological Machines

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Supplementary Text

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Characterization of Cantilever Thickness

Thicknesses were characterized by embedding 0.1% (w/v) fluorescent polystyrene beads (500 nm, Spherotech) at 1:500 dilution in the cantilevers during photopolymerization. To change the cantilever thickness, the volume of the pre-polymer solution was varied. After photopolymerization, the cantilevers were allowed to swell in PBS on a shaker overnight at room temperature. An inverted fluorescent microscope (IX81, Olympus) with IPLab (Becton Dickenson) software was used to optically measure the vertical position of the microscope stage by manually adjusting the focus on the topmost layer of beads in the gel, z_1. The vertical position was then readjusted to the bottommost layer of beads in the gel, z_2. The cantilever thickness was then calculated using: $d = z_2 - z_1$.

Determination of the Radii of Curvature

To minimize manual error in measuring the radius of curvature (ROC), we used the average of the three ROCs obtained by three independent methods (Supplementary Fig. S6). First, we used the ThreePointCircularROI plugin in ImageJ software (NIH), which automatically calculates the center of circle and the radius of curvature from the three manually-specified points on the cantilever image. Secondly, we used the arc angle of the bent cantilever, θ to calculate ROC. The center of the circle calculated with previous method was used to measure the arc angle. Since the length of the cantilever, L_c is fixed to be 4 mm, the ROC can be readily obtained with a simple equation $L_c = \theta$ *ROC. Lastly, the fitting circle was manually superimposed on the cantilever images and the radius of the circle was used.

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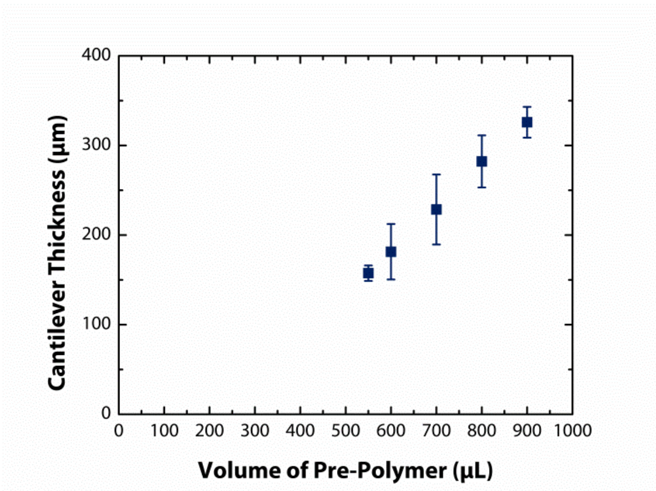
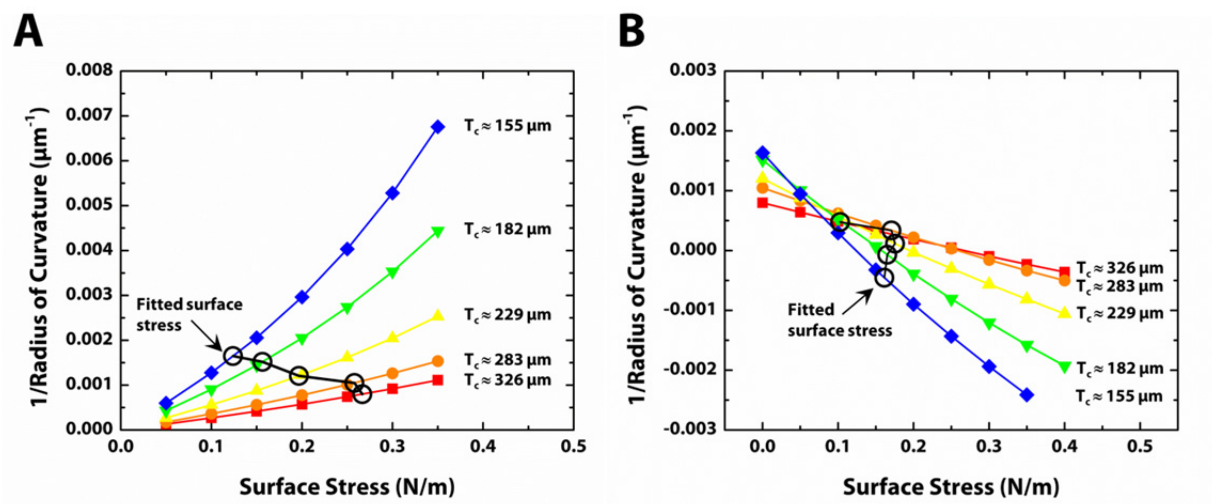
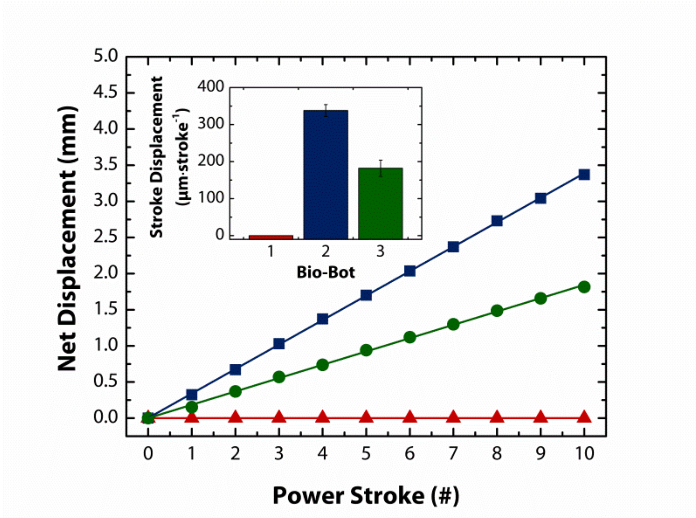


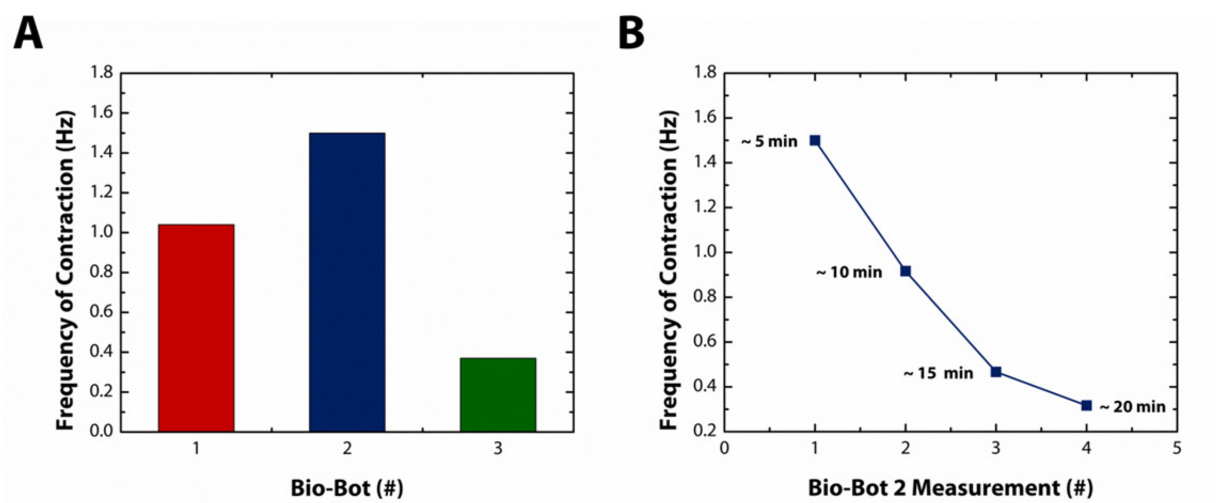
Supplementary Figure S1. Characterization of cantilever thickness. Plot of cantilever thickness vs. volume of pre-polymer solution in experimental set-up. Volumes below 550 μL did not cover build area for uniformly-thick polymerization.



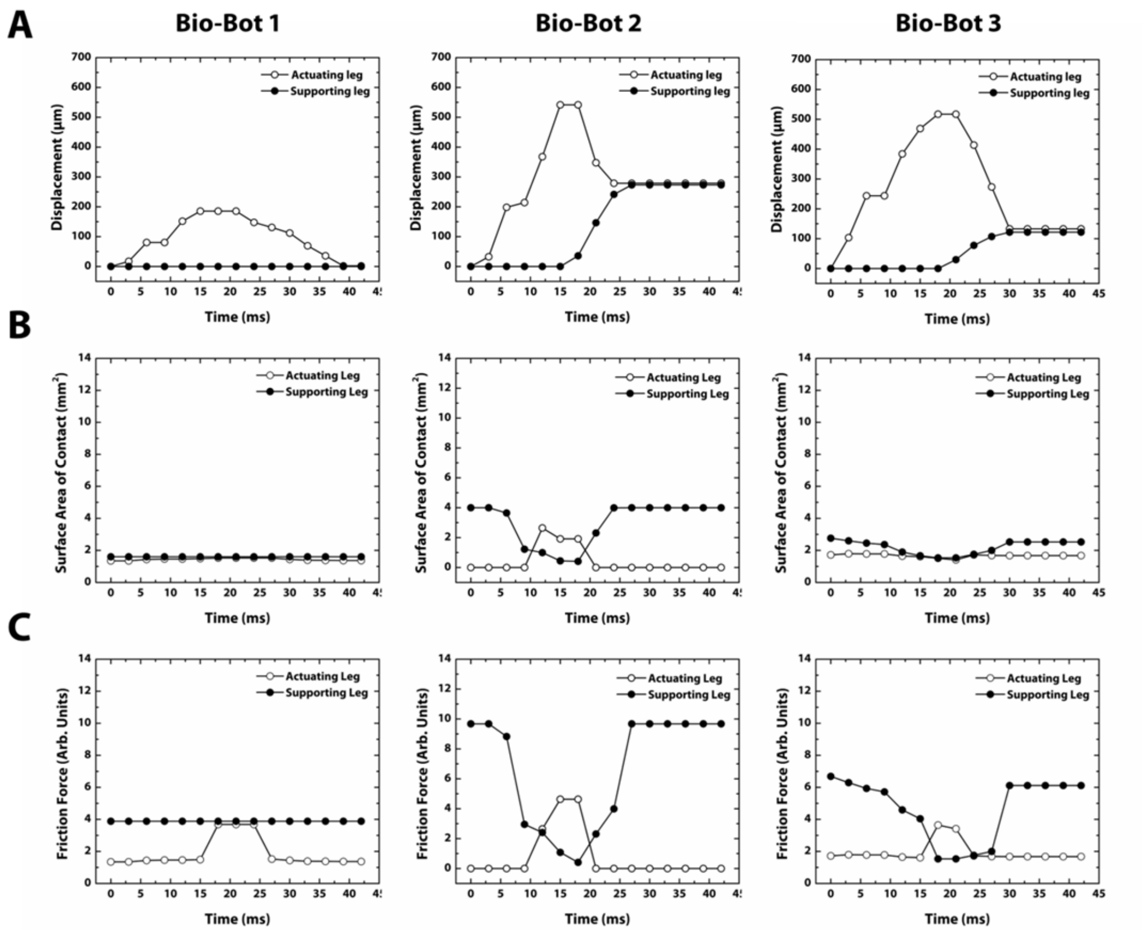
Supplementary Figure S2. Extraction of residual and cell-induced surface stresses. (**a**) Plot of inverse radius of curvature vs. surface stress for residual curvature (pre-seed). (**b**) Plot of inverse radius of curvature vs. surface stress for residual + cell-induced curvature (post-seed). The measured radius of curvature was used to interpolate residual and cell-induced surface stresses from these plots.



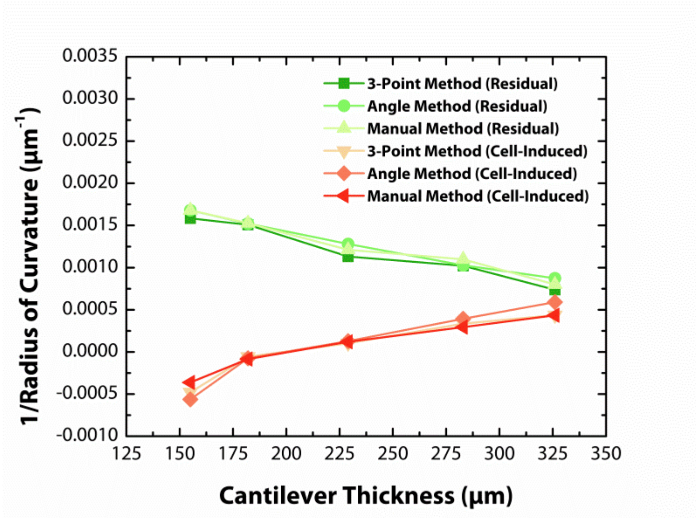
Supplementary Figure S3. Net displacement of bio-bot designs. Plot of net displacement vs. power stroke for each bio-bot design. The inset is a plot of average velocity vs. bio-bot design, which is extracted from the plot of net displacement vs. time.



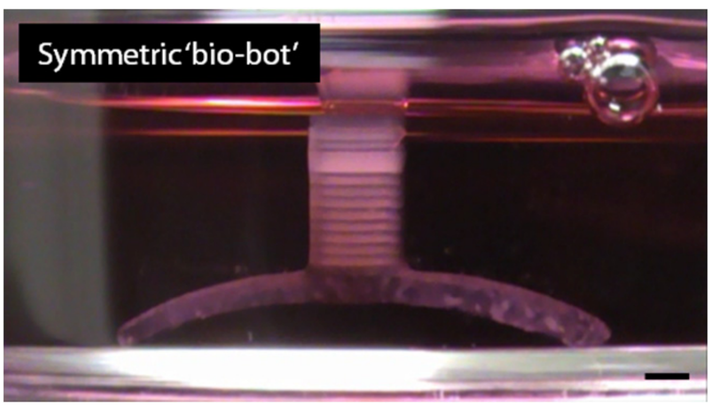
Supplementary Figure S4. Measured frequency of contraction for bio-bots. (**a**) Plot of frequency of contraction vs. bio-bot. (**b**) Plot of frequency of contraction vs. number of measurement for bio-bot 2. Each measurement took approximately 5 minutes each.



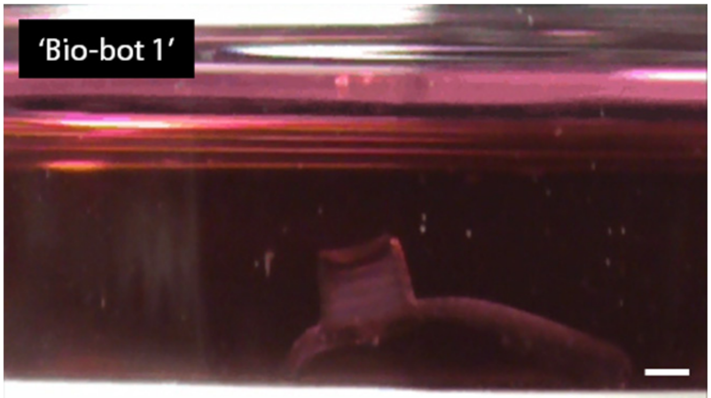
Supplementary Figure S5. Derivation of relative friction force: $F = \mu \cdot N(A)$, where μ is the coefficient of friction and $N(A)$ is the normal force as a function of the surface area of contact. (**a**) Plot of displacement vs. time for the actuating and supporting legs during a single power stroke to determine their motion state. (**b**) Plot of the surface area of contact vs. time between the legs and substrate during a single power stroke. (**c**) Plot of relative friction force vs. time by multiplying the surface area of contact by the normalized ratio of static and kinetic friction depending on the motion state of each leg.



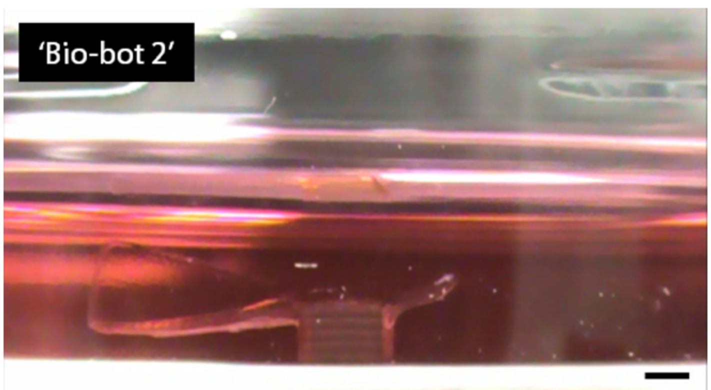
Supplementary Figure S6. Plot of inverse radius of curvature vs. cantilever thickness for residual (pre-seeded) and residual + cell-induced curvature (post-seeded). The radius of curvature was obtained by three independent methods to minimize manual error (Supplementary Text).



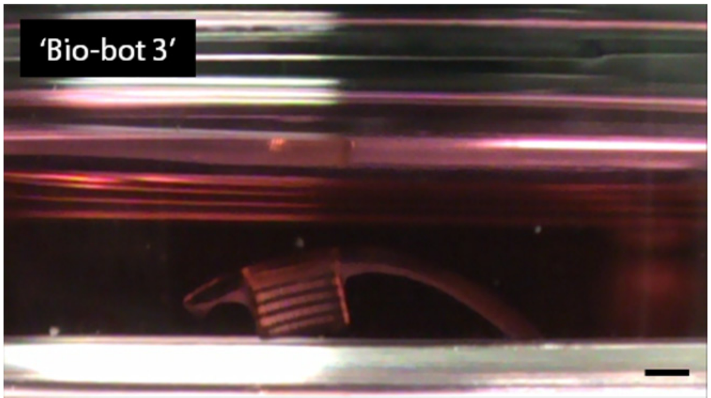
Supplementary Movie 1. Symmetric bio-bot. Side view of an actuating symmetric bio-bot on two cantilever 'legs', but resulting in no net movement after 3 days in culture. Scale bar is 1 mm.



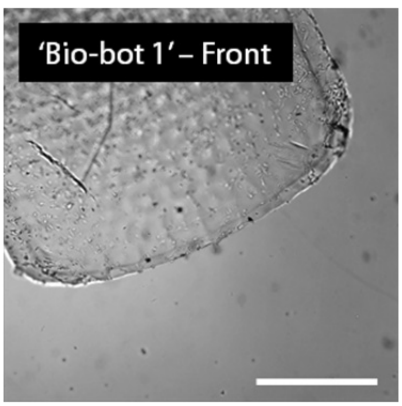
Supplementary Movie S2. Asymmetric 'bio-bot 1' (326 μm thick). Side view of an actuating asymmetric bio-bot 1 on two cantilever 'legs' with one leg shortened, but resulting in no net movement after 3 days in culture. Scale bar is 1 mm.



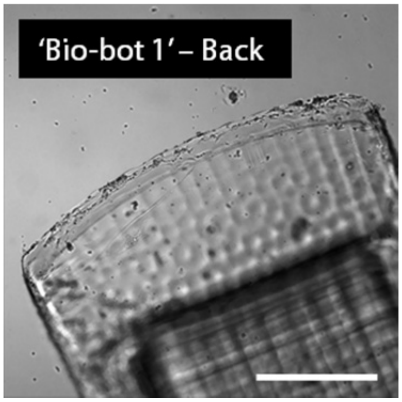
Supplementary Movie S3. Asymmetric 'bio-bot 2' (182 μm thick). Side view of an actuating asymmetric bio-bot 2 resulting in a maximum recorded velocity of ~236 $\mu m \cdot s^{-1}$, with a power stroke of ~354 μm and average beating frequency of ~1.5 Hz after 3 days in culture. Scale bar is 1 mm.



Supplementary Movie S4. Asymmetric 'bio-bot 3' (155 μm thick). Side view of an actuating asymmetric bio-bot 3 resulting in a maximum recorded velocity of ~71 $\mu m \cdot s^{-1}$, with a power stroke of ~182 μm and average beating frequency of ~0.39 Hz after 3 days in culture. Scale bar is 1 mm.



Supplementary Movie S5. Asymmetric 'bio-bot 1' (326 μm thick). Top view of the actuating leg of an asymmetric bio-bot 1 resulting in a rare instance of net movement (~27 $\mu m \cdot s^{-1}$) after 3 days in culture. Scale bar is 1 mm.



Movie S6. Asymmetric 'bio-bot 1' (326 μm thick). Top view of the supporting leg of an asymmetric bio-bot 1 resulting in a rare instance of net movement (27 $\mu m \cdot s^{-1}$) after 3 days in culture. Scale bar is 1 mm.