Supplementary Material (ESI) for Lab on a Chip

Lifespan-on-a-Chip: microfluidic chambers for performing lifelong observation of *C. elegans*

SUPPLEMENTARY INFORMATION

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Supplementary experimental procedures

Choice of culturing conditions: solid versus liquid, axenic versus monoxenic

In the laboratory, it is possible to cultivate *C. elegans* in both axenic and monoxenic media. An axenic medium is one that contains no living organism other than the organism being cultivated; a monoxenic medium is one that contains one additional organism – in the case of *C. elegans*, a monoxenic medium contains bacteria as a source of food for the worms. Typically, cultivation of *C. elegans* is on a solid support with a monoxenic food source – on nematode growth media (NGM) agar plates seeded with lawns of *E. coli*.[1] It is possible, however, to culture worms in liquid, either in a suspension of bacteria (monoxenic liquid culture)[2-4] or in a synthetic liquid medium (axenic liquid culture).[5, 6] For the development of a microfluidic lab-on-a-chip system for cultivating and studying worms, liquid medium is more suitable than solid medium, because of the number of tools available for manipulating liquids in microfluidic systems.[7] We elected to use *monoxenic* liquid medium, rather than *axenic* liquid medium, because the development of worms grown in currently available axenic formulations is significantly slower than the development of worms grown on bacteria.[6]

Fabrication of PDMS devices

We used photolithography to pattern raised features of SU-8 photoresist (Microchem Corp., Newton, MA) on a silicon wafer (Silicon Sense, Inc., Nashua, NH). This silicon master served as a template for replica molding with poly(dimethyl siloxane) (PDMS, Dow Corning Sylgard 184, Corning, NY). We treated the masters with tridecafluoro(1,1,2,2 tetrahydroooctyl) trichlorosilane (Gelest, Inc., Philadelphia, PA) by placing the masters under vacuum for three

hours along with a small volume (100 μL) of the trichlorosilane. This silanization step was necessary (i) to prevent the adhesion of PDMS to the master during the molding process, and (ii) to alter the surface energy of the master to enable the creation of domed chambers on the master. Using conventional photolithography, masters were patterned with the design shown in **Fig. 1b** with features that were uniformly 50 μm in height. To align the drop, it was necessary for the experimenter to hold the tip of a pipet (containing the 1-μL droplet of SU-8/PGMEA) directly above the 1.5-mm circle of SU-8 on the master such that when the experimenter expelled the droplet from the pipet, the droplet made contact with the 1.5-mm circle. The contact line of the SU-8/PGMEA droplet did not advance past the edge of the cured SU-8 features on the master, and surface tension prevented the droplet from advancing along the straight microchannels on either side of the circular chamber. (Masters that did not include clamps were patterned with features that were uniformly 100 μm in height.) To create the domed chambers, we added 1-μL drops of 1:1 SU-8 photoresist:propylene glycol monomethyl ether acetate (PGMEA) to the 16 circular regions of the master that would form the chambers of the device. (If masters were not silanized, the contact angle of 1:1 SU-8:PGMEA on the cross-linked SU-8 features of the master would be too low to enable the formation of domes.) We then baked the master at 55 °C for 3 hours, and exposed the master to a source of UV light to cross-link the domed features. It was not necessary to repeat the silanization of the masters prior to replica molding.

Prefabricated screw valves were fabricated as previously described.[8] We elected to use screw valves for three reasons: (i) the fabrication of screw valves is simple, (ii) unlike most valves for microfluidic systems, screw valves do not require any input of energy to remain closed or open, and (iii) because the device did not require rapid switching of valves, the manually

actuated screw valves were sufficiently fast for our application. We embedded the screw valves into the device by spreading a thin layer of PDMS prepolymer onto the master, positioning the valves in the locations indicated by **Fig. 1b**, and pouring PDMS prepolymer around the valves. The device with valves was cured overnight at 60 ºC.

Once the PDMS had cured, we removed the device from the master and punched a 1-mm inlet hole and 1.5-mm outlet holes using circular biopsy punches (Shoney Scientific Inc., Waukesha, WI). We sealed the PDMS device to a glass slide (50x75mm, 1.2 mm thick, VWR) that had been spin-coated with PDMS (1000 rpm for 45 s) and cured overnight at 60 ºC. By exposing the surfaces of the PDMS-coated glass and the PDMS device to an air plasma for one minute, we oxidized the surfaces, and rendered them hydrophilic. Immediately after plasma oxidation, we placed the PDMS device, with the patterned side facing down, onto the PDMS-coated glass slide. To ensure that the two surfaces were in conformal contact, we gently rolled the edge of a polystyrene Petri dish over the PDMS device (the conformal seal between the two surfaces is visible to the naked eye). By placing the oxidized surfaces in conformal contact, we created an irreversible, covalent seal. After sealing the surfaces together, we placed the device in an oven for 10 minutes at 60 ºC to enable the surfaces to bond completely. (Note that if the PDMS surfaces do not seal together completely, it may be necessary to adjust the length of time that the surfaces of plasma oxidation.) We then immediately filled the channels of the device with a 10% (v/v) solution of mPEG-silane in ethanol. After 8 hours, we flushed the device with at least 1 mL of fresh ethanol, and dried the device with nitrogen. We repeated this cycle of rinsing with ethanol / drying with nitrogen three times, and subsequently placed the device under vacuum for 12 hours. At this stage, the devices were ready to be filled with S medium and

loaded with worms.

Preventing bacterial adhesion with flow

In addition to treatment of the walls with mPEG, the maintenance of continuous flow was an important factor in preventing the adhesion of bacteria to the walls of the device. Shear stress on surfaces (resulting from the flow of liquid over the surface) can prevent the adhesion of bacteria and can promote the removal of adhered bacteria.[9] In our experiments, the overall volumetric rate of flow through the array of chambers was approximately 700 μL/h. Assuming that the fluidic resistance of each of the 16 segments of the array was equal, the volumetric rate of flow through each chamber would have been $1/16^{th}$ of that value (~44 μL/h, or ~0.012 μL/s). Using some simplifying approximations (fully developed, steady-state laminar flow in a parallel plate configuration), we can obtain a rough estimate the shear rate, σ, using equation (1), where Q is the volumetric flow rate, u is the average linear velocity of the fluid, h is the height of the microchannel, and w is the width of the microchannel.[10]

$$\sigma = \frac{12 \cdot u}{h} = \frac{12 \cdot Q}{h^2 w}, \text{ where } u = \frac{Q}{hw} \qquad (1)$$

For devices that were 100 μm in height, if we consider a cross-sectional slice through the center of the circular chamber, perpendicular to the direction of flow, h is equal to 100 μm and w is equal to 1500 μm. From equation (1), we obtain an estimated shear rate of 9.7 s^{-1}. Studies

examining the effect of shear rate on the adhesion bacteria to surfaces in the presence of flow have demonstrated that shear rates of 1.0 s^{-1} are sufficient to prevent the adhesion of E. coli to surfaces that have been coated with poly(ethylene glycol).[9]

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Supplementary Table S1. Summary of lifespans and features of growth for each worm

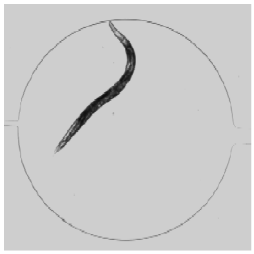
Worm	Lifespan (days)	Day of end of growth	ΔV (nL)	V_{max} (nL)	Growth rate (nL/day)
1	9	4	0.98	4.1	0.98
2	9	5	1.3	5.1	0.67
3	11	4	0.92	4.1	0.92
4	11	6	2.1	5.8	0.64
5	12	5	1.8	4.6	0.88
6	13	6	2.5	6.5	0.81
7	15	8	3.8	7.7	0.77
8	15	7	2.2	6.1	0.51
9	16	9	5.0	8.4	0.75
10	16	6	1.5	5.9	0.48
11	16	7	2.3	6.6	0.58
12	16	8	2.6	6.8	0.54
13	17	7	2.6	6.9	0.58
14	17	8	4.5	8.4	0.87
15	17	7	2.6	6.1	0.65
16	17	7	4.5	8.0	1.0
17	17	7	3.8	7.6	0.97
18	17	9	3.0	6.8	0.54
19	18	9	3.7	7.5	0.60

20	18	7	0.21	3.2	0.070

Supplemental Movie 1 The video shows the loading process for two L4 worms into two chambers. (The procedure for 16 chambers is the same.) Initially, the screw valves for both chambers are open (the valves are off-screen, above the chambers in the video). A single worm is added to the outlet of the device (off-screen, below the chambers in the video). The experimenter controls the flow of liquid in the device by attaching a fluid-filled syringe to the outlet via polyethylene tubing and manually depressing the plunger of the syringe. Clip 1 of the movie shows the experimenter guiding a worm into the chamber on the left (labeled "13"). Once the worm is in the chamber, the experimenter removes the syringe from the outlet and closes the valve associated with chamber #13. Clip 2 of the movie shows the loading of a second worm into the device. Because the valve for chamber #13 is closed, the experimenter is able to guide this second worm into the chamber on the right (labeled "14") without disturbing the position of the first worm.

Supplementary Fig. S1 Procedure for analyzing size and swimming frequency in *C. elegans*. Each day, beginning on day 3, we made 1-min video-recordings of each worm within the device. We extracted frames from daily video recordings of the worms, and applied a series of morphological algorithms using MATLAB® to calculate the area, length, and body angle, θ, for each worm. The volume of the worm was estimated as the volume of a cylinder with the same cross-sectional area and length as the worm. The swimming frequency was determined by measure the number of oscillations in θ per unit time.

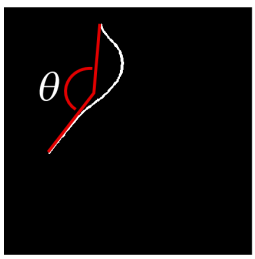
Supplementary Fig. S1



1. convert each frame to binary image
2. measure cross-sectional area of worm

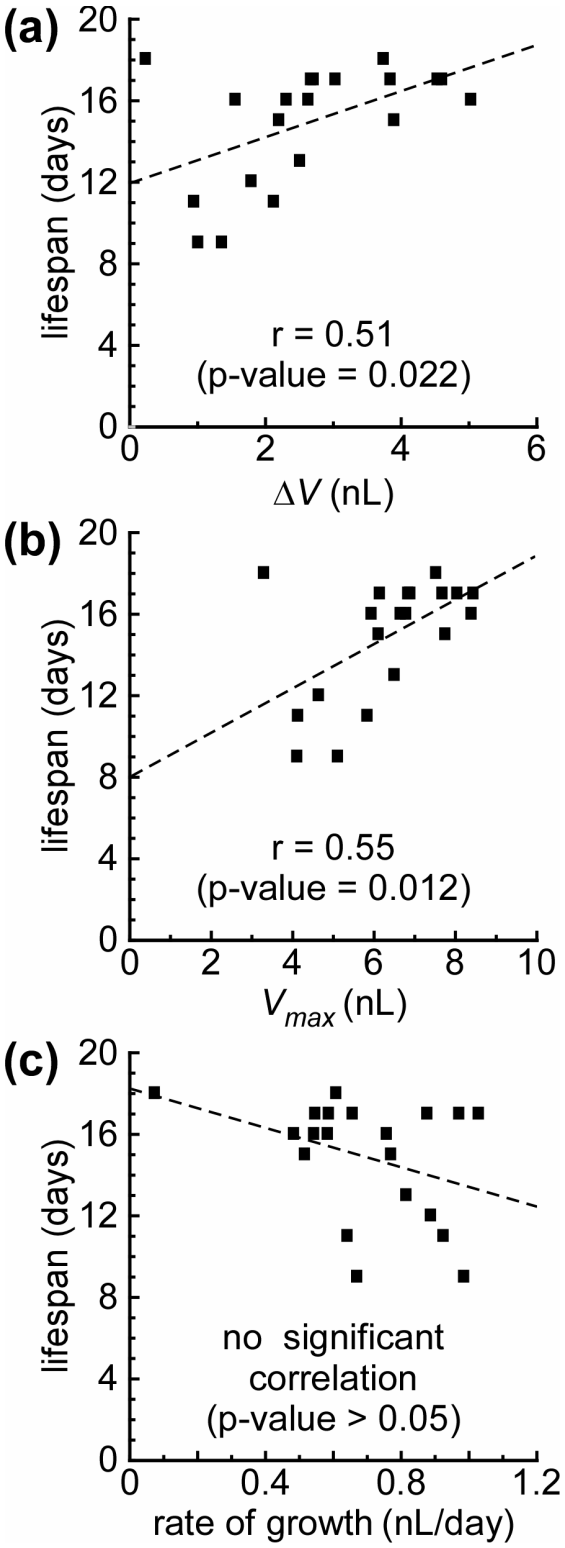
1. reduce the body of the worm to a single line
2. measure length of worm

1. identify head and tail ends
2. identify center of mass
3. measure body angle, θ



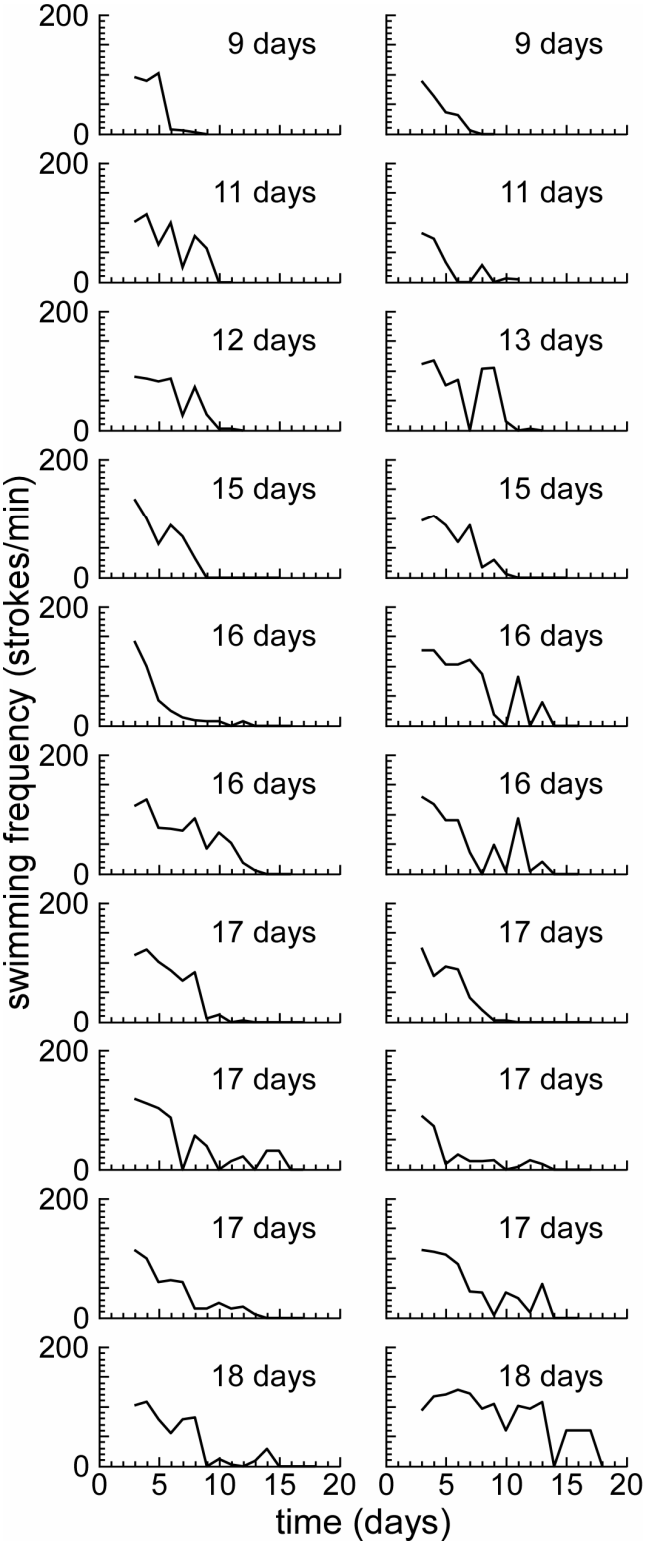
Supplementary Fig. S2 Features of the growth phase correlate with lifespan. Scatter plots comparing lifespan with **(a)** the change in volume of each worm from day 3 to the end of growth, ΔV, **(b)** the volume at the end of the growth phase, $V_{\max}$, and **(c)** the growth rate during the growth phase (N = 20 worms). Each plot displays *r*, the Pearson correlation coefficient, and the corresponding *p-value*. The dashed lines represents estimated regression lines, obtained using the method of least squares.

Supplementary Fig. S2



Supplementary Fig. S3 The age-related decline in swimming frequency for each worm. Each plot represents the swimming frequency over time for an individual worm. The lifespan of each worm is indicated in the upper-right corner of each plot.

Supplementary Fig. S3



Supplementary Fig. S4 Age related decline in swimming frequency correlates with lifespan. **(a)** A scatter plot comparing the swimming span for a threshold of 20 strokes/min with lifespan for each animal in the population. **(b)** A scatter plot comparing the swimming span for a threshold of 30 strokes/min with lifespan for each animal in the population (N = 20 worms). Each plot displays *r*, the Pearson correlation coefficient, and the corresponding *p-value*. The dashed lines represents estimated regression lines, obtained using the method of least squares.

Supplementary Fig. S4