Electronic Supplementary Material (ESI) for Reaction Chemistry & Engineering.

Supplementary Information

Microfluidic Localized Hydrogel Polymerization Enables Simultaneous Recording of Neural Activity and Behavior in *C. elegans*

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1. Effect of prolonged exposure to prepolymer solution

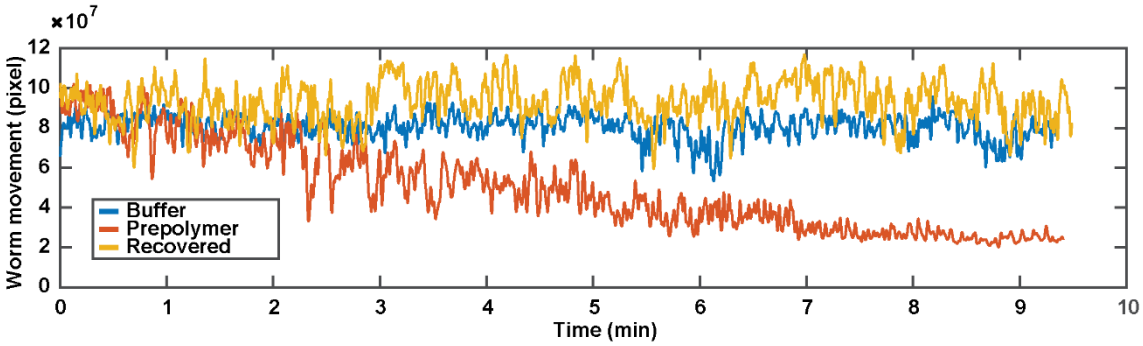

Figure S1. *C. elegans* motion activity under prolonged exposure to the hydrogel prepolymer solution. The motion activity was quantified by computing pixel differences between consecutive frames of a movie recording three size-controlled worms swimming in buffer (blue and yellow lines) or prepolymer (orange line). They were placed in a well to keep their motion in the microscopic field of view. The animal experiences slow plasmolysis due to higher osmotic pressure of the prepolymer solution (30% PEGDA). The movement is restored to normal level when the animals are recovered in buffer after 10 mins of prepolymer exposure.

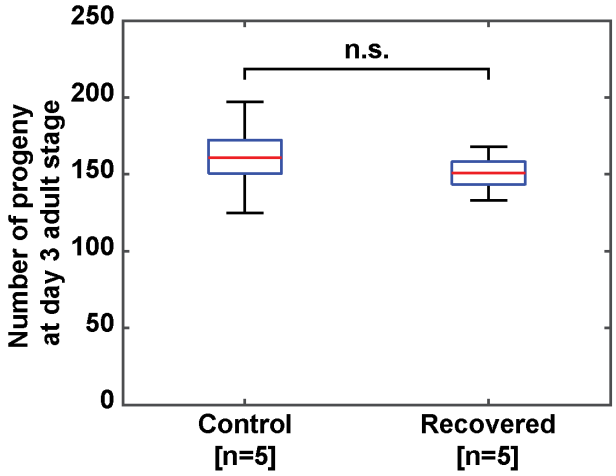

Figure S2. Number of progenies from animals recovered from polymerized hydrogel compared to those not exposed to hydrogel. The recovered animals were initially immobilized in hydrogel for

at least 10 minutes before being recovered and transferred to culture agar plates. The number of larval progenies was counted at day 3 adult stage (approximately 65 hours post L4 stage). For the box plot, the central mark indicates the median, and the outline indicates the lower and upper quartiles. For each box plot, the center line indicates the median, and the bottom and top edges indicate the 25th and 75th percentiles, respectively. The whiskers indicate the most extreme data points that are not outliers.

2. Damköhler number derivation

The change in oxygen concentration in a reaction volume is described by the following mass balance

$$\frac{\partial[O_2]}{\partial t} = [O_2]\frac{\partial V_x}{\partial x} + D_O\frac{\partial^2[O_2]}{\partial z^2} - k_O[O_2][R]$$

where D_o is diffusivity of oxygen, k_O is the rate constant of oxygen inhibition, and [R] is the concentration of radical species.

The competing relationship between the consumption and replenishment rate of reaction-inhibiting oxygen is described by a Damköhler number. It is defined as the ratio of the rate at which oxygen is quenched by the reaction to the rate at which oxygen is supplied by convective flow. By non-dimensionalizing the variables from the mass balance, the Damköhler number is expressed as

$$Da = \frac{rate\ of\ reaction}{rate\ of\ convection} = \frac{k_O[O_2]_{eq}[R]_{eq}}{[O_2]_{eq}\frac{\partial Vx}{\partial x}} = \frac{k_O[R]_{eq}}{\frac{\partial Vx}{\partial x}} = \frac{k_O[R]_{eq}L}{U_x}$$

The characteristic length L is the diameter of the UV mask, and U_x is the flow speed along the direction of the microfluidic channel.

To estimate the concentration of radical species, quasi-steady-state approximation is used with the assumption that the rate of radical generation is equal to the rate of radical consumption.

$$Rate\ of\ radical\ generation\ (r_a) = Rate\ of\ radical\ consumption\ (r_c)$$

$$r_a = \varphi\varepsilon[PI]I_0e^{-\varepsilon[PI]H}$$

Here φ is the quantum yield of radical formation of the photoinitiator, ε is the molar extinction coefficient of the photoinitiator at 390 nm, [PI] is the molar concentration of the photoinitiator, I_0 is the initial intensity of the UV source, and H is the height of the microfluidic channel.

$$r_c = k_t[R]^2 + k_O[R][O_2]$$

k_t is the rate constant of the radical termination reaction (a radical reacting with another radical). k_O is the rate constant of the oxygen inhibition reaction (a radical reacting with oxygen).

Solve for [R]

$$[R]=\frac{-k_o[O_2]+\sqrt{(k_o[O_2])^2+4r_ak_t}}{2k_t}$$

Multiply the numerator and denominator by $\sqrt{(k_o[O_2])^2+4r_ak_t}+k_o[O_2]$

$$=\frac{4r_ak_t}{2k_t\left(\sqrt{(k_o[O_2])^2+4r_ak_t}+k_o[O_2]\right)}$$

$$Since\ (k_o[O_2])^2 \gg 4r_ak_t$$

$$=\frac{4r_ak_t}{2k_t(2k_o[O_2])}$$

$$=\frac{r_a}{k_o[O_2]}$$

$$Da=\frac{k_or_aL}{k_o[O_2]_{eq}U_x}=\frac{r_aL}{[O_2]_{eq}U_x}$$

3. Example Damköhler number calculation

$$r_a=\varphi\varepsilon[PI]I_0e^{-\varepsilon[PI]H}$$

$$=(0.6)\cdot\left(25\ \frac{m^3}{mol\cdot m}\right)\cdot\left(170\ \frac{mol}{m^3}\right)\cdot\left(2.6\cdot10^{-2}\frac{mol}{m^2\cdot s}\right)\cdot e^{-\left(25\ \frac{m^3}{mol\cdot m}\right)\cdot\left(170\ \frac{mol}{m^3}\right)\cdot(7\cdot10^{-5}m)}$$

$$=49.2\frac{mol}{m^3\cdot s}$$

$$Da=\frac{r_aL}{[O_2]_{eq}U_x}$$

$$=\frac{\left(49.2\ \frac{mol}{m^3\cdot s}\right)\cdot(2\cdot10^{-4}\,m)}{\left(1.5\ \frac{mol}{m^3}\right)\cdot\left(3.67\cdot10^{-4}\ \frac{m}{s}\right)}$$

$$=17.9$$

Table S1. Parameters used for Damköhler number estimation

Symbol	Definition	Value	Units	Source
D_O	Diffusion coefficient of oxygen	$2.84*10^{-11}$	m^2/s	[1]
H	Height of channel	$7*10^{-5}$	m	measured
I_0	Initial light intensity	$2.6*10^{-2}$	$E/(m^2\ s)$	measured
k_O	Rate constant of oxygen inhibition	$5*10^5$	$m^3/(mol\ s)$	[1]
L	Diameter of circular UV mask	$2*10^{-4}$	m	measured
U_x	Flow speed	varying	m/s	measured
$[O_2]_{eq}$	Equilibrium oxygen concentration	1.5	mol/m^3	[1]
[PI]	Photoinitiator concentration	170	mol/m^3	measured
ε	Molar extinction coefficient	25	$m^3/(mol\ m)$	[2]
φ	Quantum yield of radical formation	0.6	-	[1]

4. Temporal resolution of chemical stimulus delivery in the microfluidic device

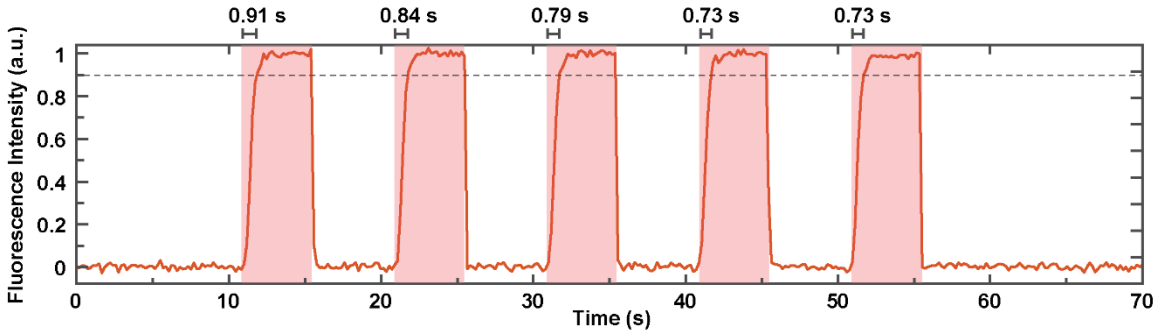

Figure S3. Temporal resolution of chemical stimulus delivery to a head immobilized *C. elegans*. Five 5s step pulses of a chemical stimulus with a fluorescent tracer were delivered to a partially immobilized worm in the microfluidic device (shaded regions). The tracer was added to visualize and quantify the stimulus delivery. The average fluorescence intensity of a segmented area in the stimulus channel was extracted for each time frame in Supplementary video 3. Temporal resolution was defined as the time required for the fluorescence intensity to reach 90% of its maximum intensity. All five pulses of the stimulus exhibited a temporal resolution of less than a second.

Supplementary videos

Video S1- Operation of the microfluidic device to partially immobilize *C. elegans*. Play speed: 1x.

Video S2- Simultaneous recording of neuronal activity and behavior. The image brightness and contrast are modified to allow visualization of animal's auto-fluorescent body. Play speed: 2x.

Video S3- Chemical stimulation of head-immobilized *C. elegans*. The image brightness and contrast are modified to allow visualization of the fluorescent tracer in the stimulus channel of the microfluidic device. Play speed: 2x.

References

1 D. Dendukuri, P. Panda, R. Haghgooie, J. M. Kim, T. A. Hatton and P. S. Doyle, *Macromolecules*, 2008, **41**, 8547–8556.

2 B. D. Fairbanks, M. P. Schwartz, C. N. Bowman and K. S. Anseth, *Biomaterials*, 2009, **30**, 6702–6707.