ADVANCED MATERIALS TECHNOLOGIES

Supporting Information

for *Adv. Mater. Technol.,* DOI: 10.1002/admt.202201274

Creating 3D Objects with Integrated Electronics via Multiphoton Fabrication In Vitro and In Vivo

Sara J. Baldock, Punarja Kevin, Garry R. Harper, Rebecca Griffin, Hussein H. Genedy, M. James Fong, Zhiyi Zhao, Zijian Zhang, Yaochun Shen, Hungyen Lin, Catherine Au, Jack R. Martin, Mark D. Ashton, Mathew J. Haskew, Beverly Stewart, Olga Efremova, Reza N. Esfahani, Hedley C. A. Emsley, John B. Appleby, David Cheneler, Damian M. Cummings, Alexandre Benedetto, and John G. Hardy**

Supporting Information

Creating 3D objects with integrated electronics via multiphoton fabrication *in vitro* and *in vivo*

Sara J. Baldock, Punarja Kevin, Garry R. Harper, Rebecca Griffin, Hussein H. Genedy, M. James Fong, Zhiyi Zhao, Zijian Zhang, Yaochun Shen, Hungyen Lin, Catherine Au, Jack R. Martin, Mark D. Ashton, Mathew J. Haskew, Beverly Stewart, Olga Efremova, Reza N. Esfahani, Hedley Emsley, John B. Appleby, David Cheneler, Damian M. Cummings, Alexandre Benedetto and John G. Hardy**

Table S1. *In silico* toxicity screening.

Chemical	Simplified molecular-input line-entry system (SMILES) notations for species analyzed.	Safety Data Sheet	Derek Nexus Flags	Sarah Nexus Flags	Reference
PY	C1=CC=CN1	Toxic, corrosive.	Non-sensitizer of skin and non-mutagenic.	Non-mutagenic	[73]
PPY	C1(=CC=C(N1)C2=CC=C(N2)C3=CC=C(N3)C4=CC=C(N4)C5=CC=C(N5)C6=CC=C(N6)C7=CC=C(N7)C8=CC=C(N8)C)C	Not a hazardous substance or mixture according to Regulation (EC) No. 1272/2008.	Non-sensitizer of skin and non-mutagenic.	Non-mutagenic	[73]
PSS	CCC(C)C1=CC=C(C=C1)[S-](=O)(=O)=O	Not a hazardous substance or mixture according to Regulation (EC) No. 1272/2008.	Non-sensitizer of skin and non-mutagenic.	Non-mutagenic	[73]
PDMS	O([Si@](O[Si@@](C)(C)C)(C)C)[Si@](C)(C)C	Not a hazardous substance or mixture according to Regulation (EC) No. 1272/2008.	Potentially hepatotoxic and nephrotoxic.	Non-mutagenic	[75]
Irgacure D2959	C(COC1=CC=C(C=C1)C(C(C)(C)O)=O)O	May be toxic at high enough concentration.	Non-sensitizer of skin and non-mutagenic.	Non-mutagenic	[87]
PEG	OCCOCCO	Not a hazardous substance or mixture according to Regulation (EC) No. 1272/2008.	Non-sensitizer of skin and non-mutagenic.	Non-mutagenic	New
PEGDMA	C=C(C(OCCOCCOCCOCCOCCOCCOCCOC(C(=C)C)=O)=O)C	Eye irritant, skin sensitiser, toxic.	Potentially hepatotoxic and nephrotoxic; plausible chromosome damage, plausible irritant of eyes/skin, plausible sensitizer of skin; non-mutagenic.	Non-mutagenic	New
CSA	C1(C2C(CC(C1)C2(C)C)=O)CS(=O)(=O)O	Toxic, corrosive.	Non-sensitizer of skin and non-mutagenic.	Non-mutagenic	New

Table S2. Processes, parameters and corresponding thicknesses for coating glass coverslips with PDMS using the spin coater.

Conditions	Step 1 parameters (spin speed rpm, time seconds)	Step 2 parameters (spin speed rpm, time seconds)	Thickness (μm)
1	500 rpm, 30 s	500 rpm, 90 s	>75
2	500 rpm, 30 s	750 rpm, 90 s	>70
3	500 rpm, 30 s	1000 rpm, 60 s	50-60
4	500 rpm, 30 s	1000 rpm, 90 s	45-55
5	500 rpm, 30 s	1000 rpm, 120 s	40-50
6	500 rpm, 30 s	1500 rpm, 120 s	25-35
7	500 rpm, 30 s	2000 rpm, 120 s	15-25
8	500 rpm, 30 s	6000 rpm, 120 s	2-8

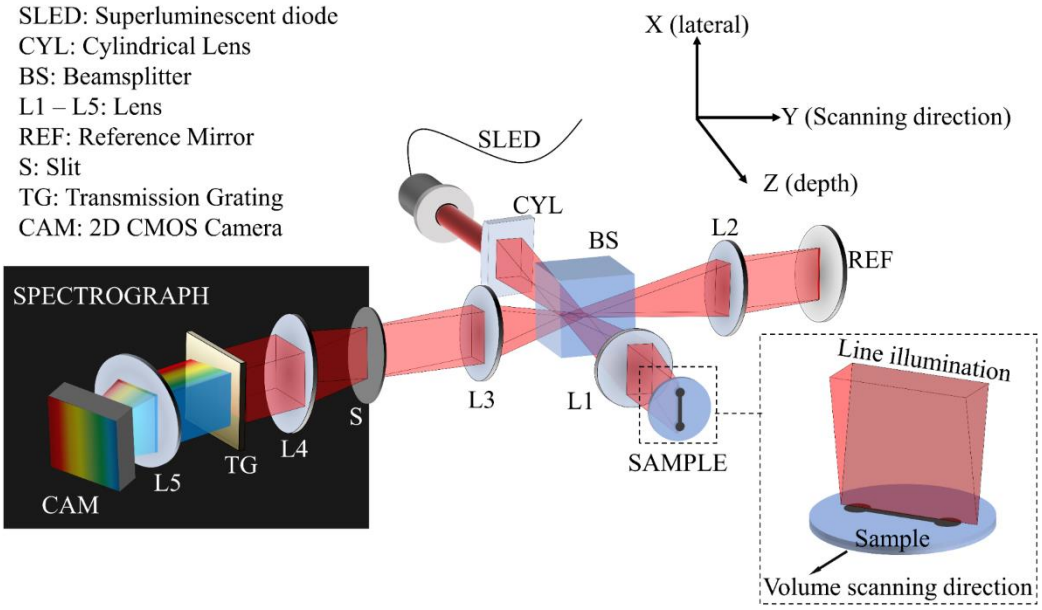


Figure S1. A schematic of the LF-OCT system used to resolve the sub-surface features of the samples. Inset shows the line-field illumination across the sample.

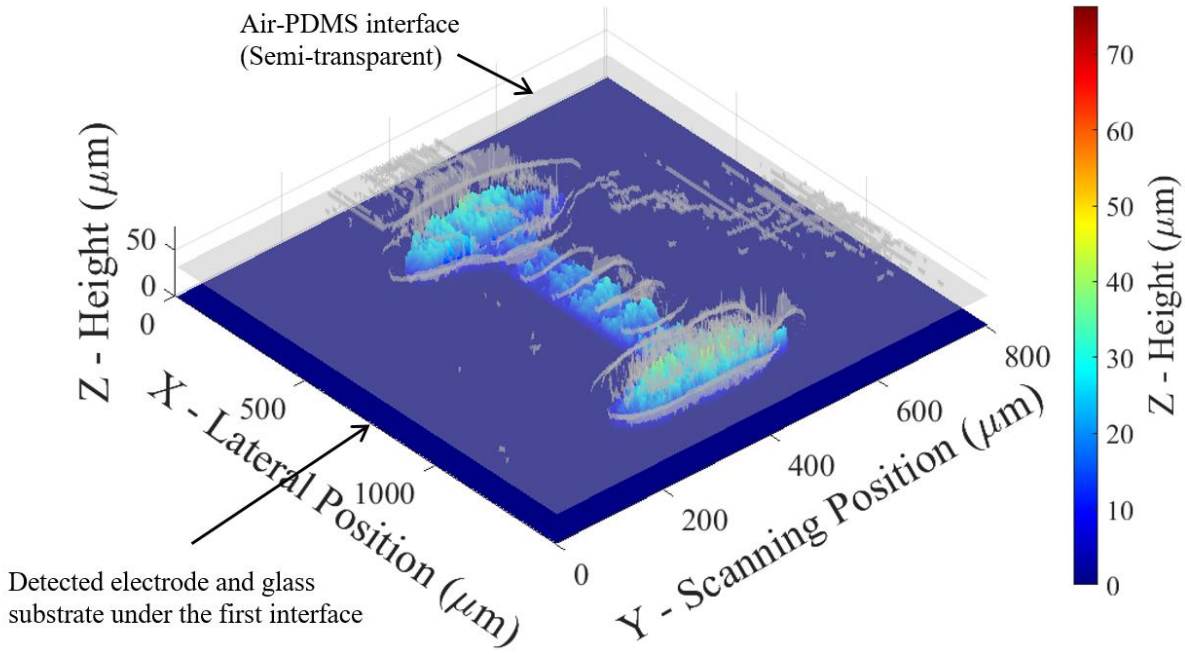


Figure S2. 3D rendering of the sample based on LF-OCT measurements showing the air-PDMS interface and the electrode and glass sub-surface layer.

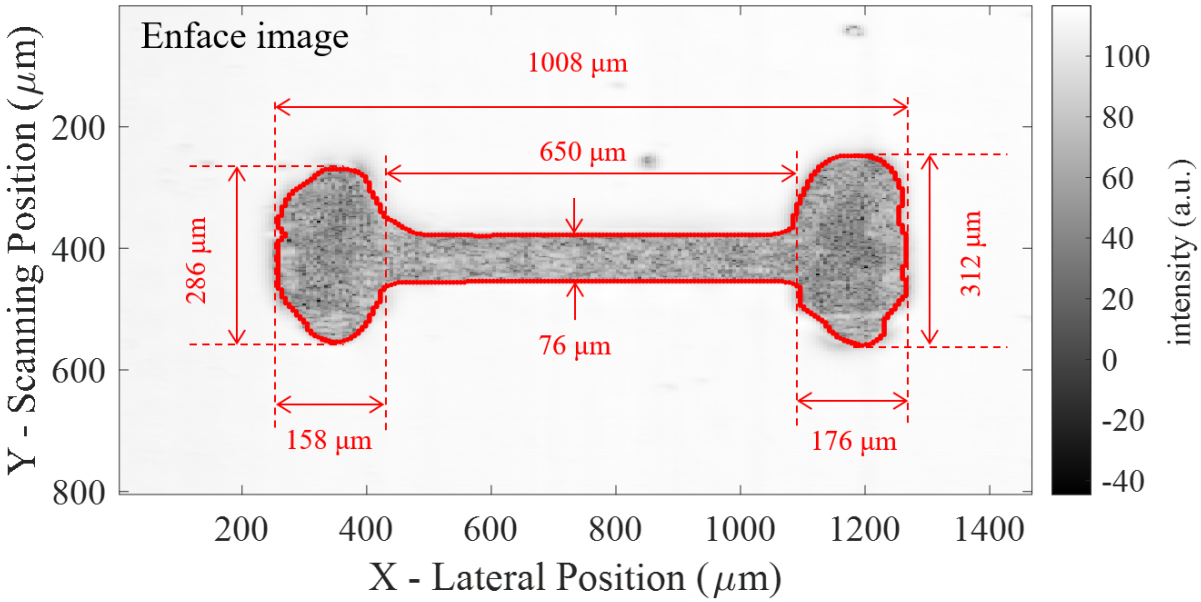
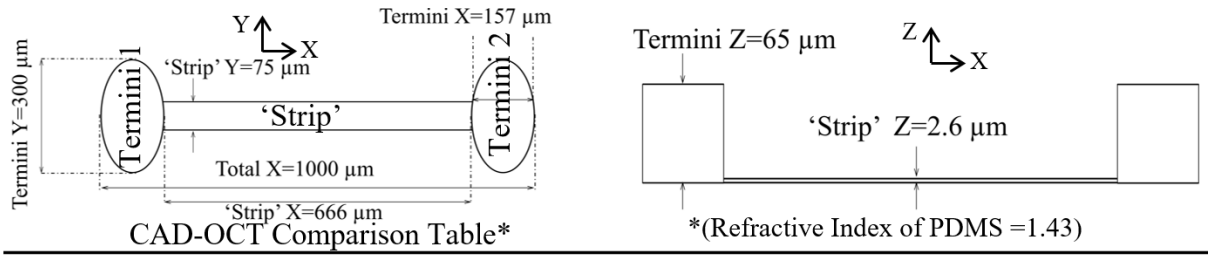


Figure S3. An enface image of the sample across the X-Y plane showing the sample topography where segmented boundary between the electrodes and substrates are outlined in red for feature size estimation.



CAD-OCT Comparison Table*

Terms	CAD	OCT	Fidelity
Termini 1 Y	300 µm	286 µm	95.3 %
Termini 2 Y	300 µm	312 µm	104 %
Termini 1 X	157 µm	158 µm	100.6 %
Termini 2 X	157 µm	176 µm	112 %
'Strip' Y	75 µm	76 µm	101 %
'Strip' X	666 µm	650 µm	97.6 %
Total X	1000 µm	1008 µm	100.8 %
Termini 1 mean Z	65 µm	Mean:17.5 ± 7.9 µm Max:42.5 Min: 0 µm	26.9 ± 12.2 %
Termini 2 Mean Z	65 µm	Mean:20.4 ± 9.2 µm Max:48.6 Min: 0 µm	31.4 ± 14.2 %
'Strip' Mean Z	2.6 µm	Mean:13.9 ± 5.9 µm Max:33.4 Min: 0 µm	535 ± 227 %

Figure S4. A comparison of CAD dimensions used for deign against OCT measurements of the printed samples with a PDMS refractive index of 1.43 at a wavelength of 840 nm.

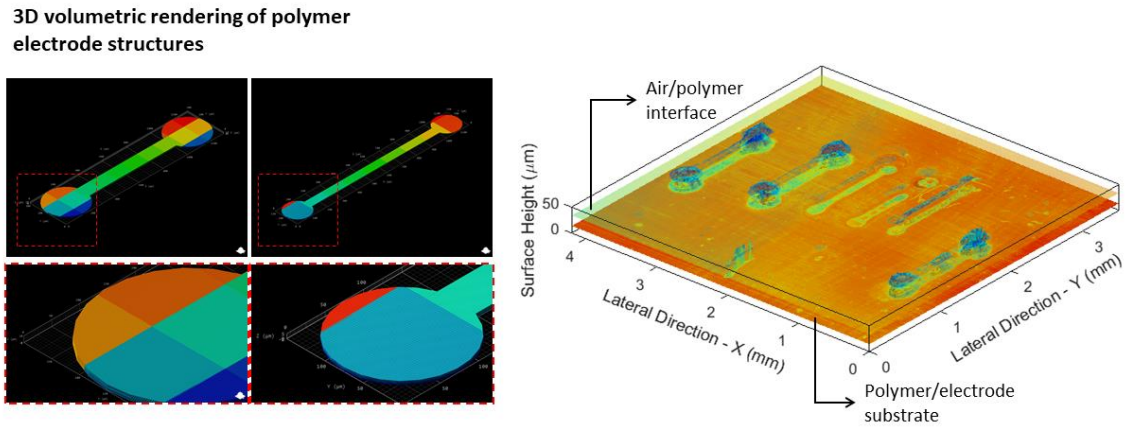


Figure S5. 3D rendering of the entire sample based on LF-OCT measurements containing all the printed structures.

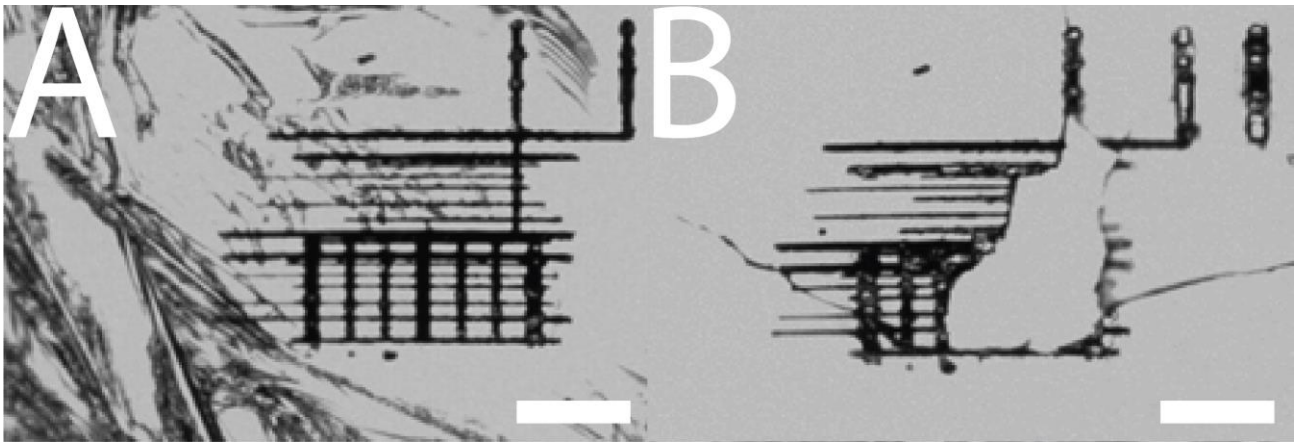


Figure S6. Pictures of PPY structures printed inside SMP films. Photos taken with LuCAM software and a x10 Primo Star plan achromat ZEISS lens attached to an optical microscope. A) Before ethanol washing. B) After ethanol washing. Scale bars represent 100 µm.

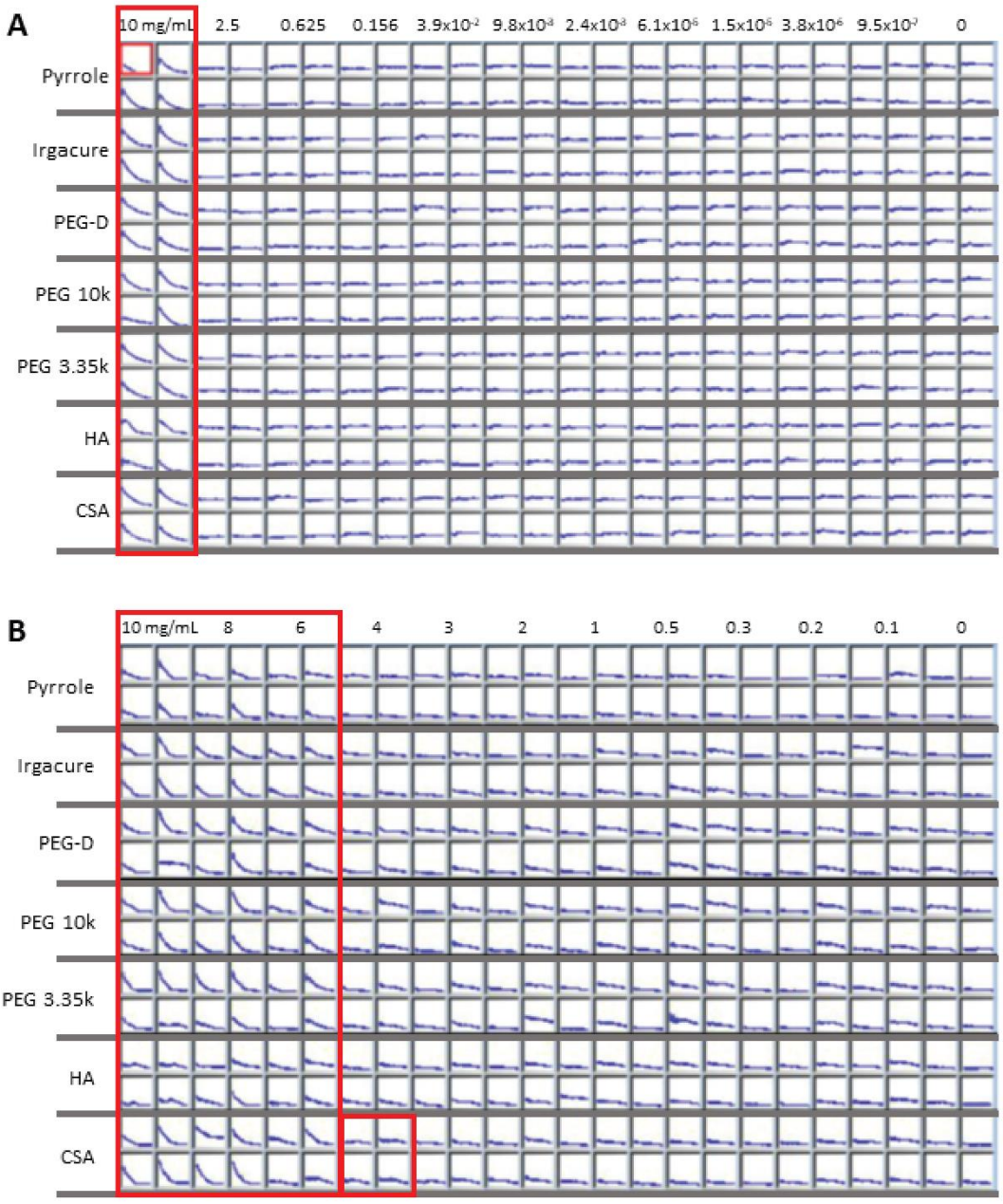


Figure S7. Photoresist components are acutely toxic to *C. elegans* above 6mg/mL. *C. elegans* day 1 adult worms are exposed to photoresist components in dilutions ranging from 10 mg/mL down to 95 ng/mL in M9 buffer and subjected to LFASS assays (Benedetto et al. 2019). (A) 24h survival assays. Concentrations below 2.5mg/mL do not kill adult worms within 24h, while 10 mg/mL of either compound kills worms within the first hour of exposure. (B) Concentrations of 6 mg/mL and above are lethal to day 1 adult worms within 24h for all compounds. 4 mg/mL of CSA is also lethal within 24h. PEG-D: Polyethylene glycol diacrylate 2,000 MW, PEG 10k: PEG 10,000 MW, PEG 3.35k: PEG 3,350 MW, HA: hyaluronic acid, CSA: camphor-10-sulfonic acid. Red boxes highlight lethal concentrations. Death is revealed by peaks or early sharp declines in blue fluorescence.

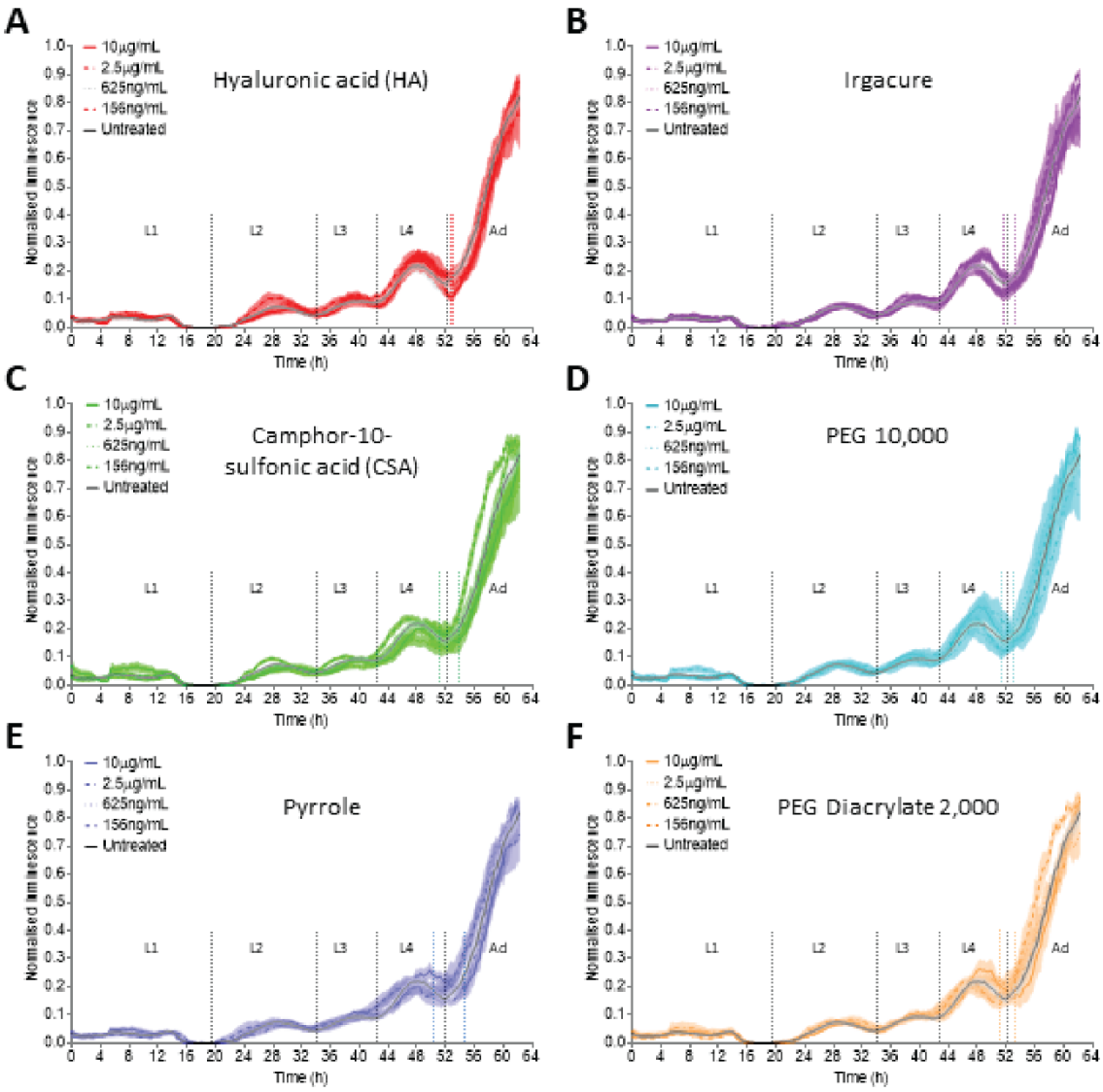


Figure S8. *C. elegans* development might be mildly affected by dilutions of photoresist components. (A-F) 10 mg/mL concentrations of either compound may lead to a mild developmental delay (0.5-2.5h out of a 51.5-54.5h needed to reach adulthood in this assay). (C-F) Conversely, 156 ng/mL of CSA, Pyrrole and PEG D may lead to slightly accelerated development (0.25-2h faster). Each developmental curve represents the average of 6-8 cohorts of 20-30 worms each except for the untreated control curve, which represent the average of 30 cohorts of 20-30 worms each. Error bars represent the standard error of the mean (SEM). L1-L4 indicate the successive larval stages. Ad: adult stage. Black dotted vertical lines indicate transitions between developmental stages for the untreated control. Colored vertical dotted lines indicate the earliest (156 ng/mL) and latest (10 mg/mL) L4 to adult transition for corresponding compounds. All conditions tested allowed successful development of worms into gravid adults. The developmental assay was adapted from Olmedo et al. 2015.

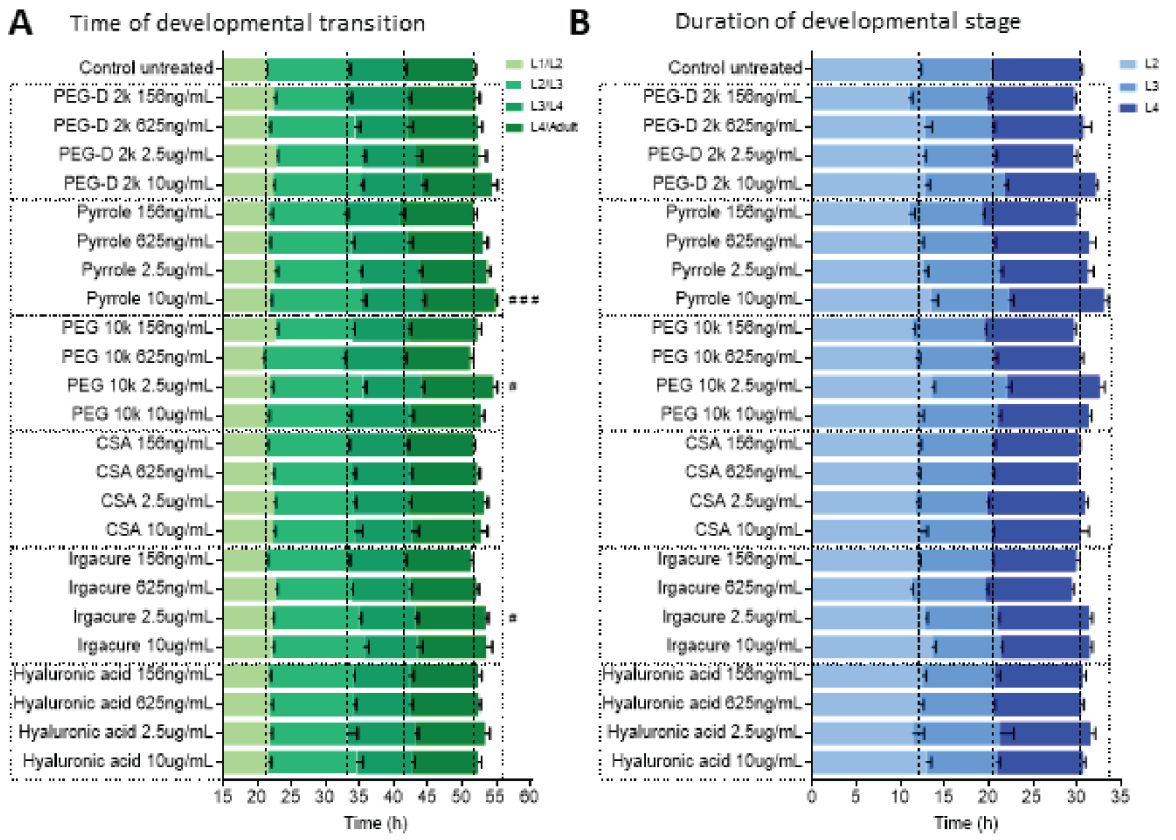


Figure S9. Quantification of *C. elegans* development phases upon exposure to dilutions of photoresist components. (A) Transition from L4 to adulthood is significantly but modestly delayed upon exposure to 10 mg/mL Pyrrole, 2.5 mg/mL, PEG 10,000, and 2.5 mg/mL Irgacure. (B) The duration of individual developmental phases is not significantly affected by any of the concentrations tested for either of the six components of the photoresist mix. L1 stage duration cannot be accurately determined in this assay as animals are synchronised post hatching before running the assay. Each developmental curve represents the average of 6-8 cohorts of 20-30 worms each except for the untreated control curve, which represent the average of 30 cohorts of 20-30 worms each. Error bars represent the standard error of the mean (SEM). L1-L4 indicate the successive larval stages. Ad: adult stage. Black dashed vertical lines indicate transitions between developmental stages for the untreated control. Significance of effects was assessed by two-way ANOVA followed by post-hoc Dunnett's test for multiple comparisons. #: $p<005$, ###: $p<0.001$. The developmental assay was adapted from Olmedo et al. 2015.

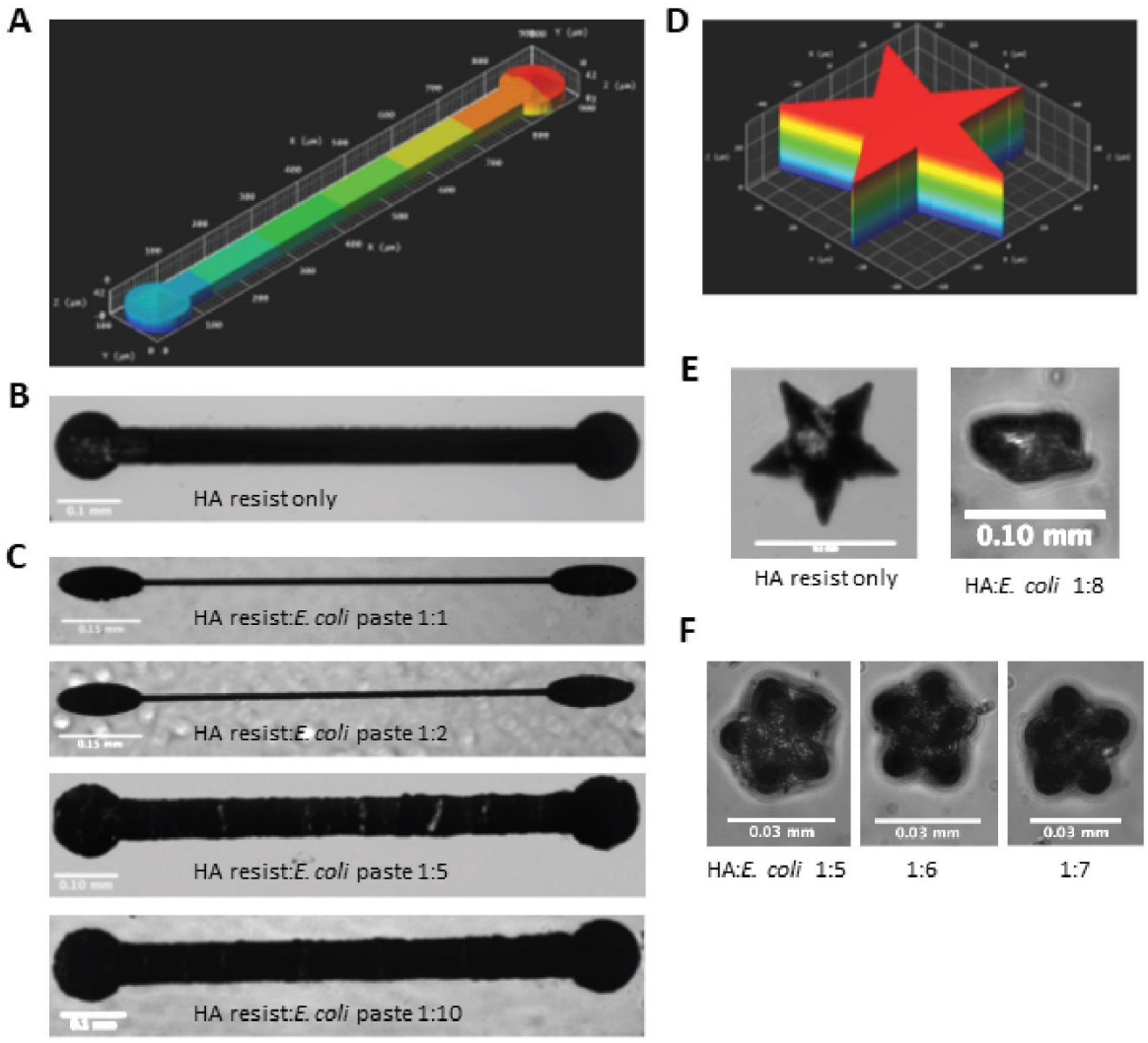


Figure S10. Optimization of resist formulation for in vivo printing. (A) CAD of mm size structures to be printed by Nanoscribe using hyaluronic acid (HA)-based formulations. (B) Printed structure from HA-based resist (HA:Irgacure:Pyrrole:PEG:PEG-D at 50:50:1:1:1) on a polydimethylsiloxane (PDMS)-coated glass coverslip. (C) Results of successive printing attempts with the same resist mixed with *E. coli* OP50 bacterial paste (100x OD_{600}=1) in various ratios. Printing of ~100mm-1mm-sized structures is still achievable with 11 times diluted HA-based resist. (D) CAD of ~10s of mm size star shape to be printed by Nanoscribe using hyaluronic acid (HA)-based formulations. (E) Printed star shapes with the same HA-based resist on polydimethyl siloxane (PDMS)-coated glass coverslip, pure (left) and diluted 9 times with bacterial paste (right). The 9 times-diluted resist cannot be printed into define shapes at of ~10s of mm scales. (F) Printing attempts of 30 mm span star shapes at 1:5, 1:6 and 1:7 HA-resist to bacterial paste ratios yield eroded star shapes with poor definition.

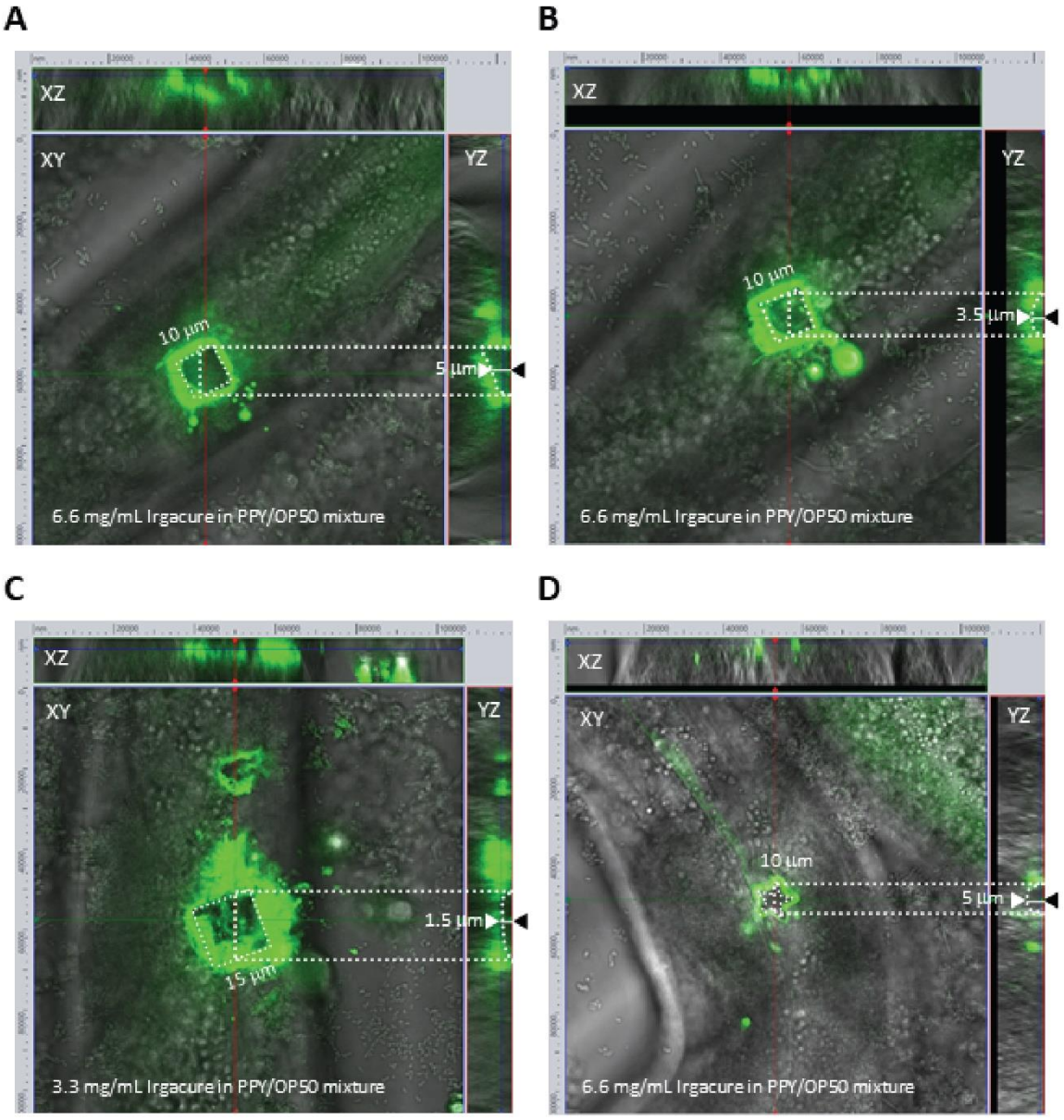


Figure S11. Semi-quantitative evaluation of printing precision on live *C. elegans* worms by confocal microscopy. (A-B) Printing of squares of 10 mm side on live *C. elegans* adults, when photoinitiator (Irgacure) is mixed at 6.6 mg/mL. Partly polymerised photoresist fluoresces in green upon 488 nm laser excitation, while fully polymerised resit appears dark, which allows visualisation of printed shapes by confocal fluorescence microscopy. Fully polymerised structures match intended dimensions with a ~10% precision, but corners are rounded, and shape are surrounded by a halo of incompletely polymerised resist extending shapes by a further 10-20%. (C) Printing of squares of 10 mm side on live *C. elegans* adults, when photoinitiator (Irgacure) is mixed at 3.3 mg/mL. Reducing curing agent concentration leads to a large unpatterned halo of partially polymerised photoresist (green smear around square shape) and incomplete curing within the intended shape boundaries (bottom left corner

of square). (D) Printing of a 10 mm star in live *C. elegans* adults, when photoinitiator (Irgacure) is mixed at 6.6 mg/mL. Fully polymerised matches intended dimensions with a ~10% precision, corners are rounded, and the thickness of the halo of incompletely polymerised resist remains within 10-20% of shape size. Target printing depth was set at 10 mm but the depth of printed structures achievable is restricted by the thickness of photoresist layer in/on the worm, which is not controlled in these experiments. Worms are exposed to a mixture of 1:5 photoresist mix (50 mg HA, 50 or 100 mg Irgacure, 1 mL PEG, 1mL Pyrrole, 1 mL PEG diacrylate) and concentrated *E. coli* OP50.