Supplemental Information

# Precise Micronscale Polymeric Networks through Piezoelectronic Inkjet Printing

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## EXPERIMENTAL

**Materials** All reaction solvents used were HPLC quality and purchased form Sigma Aldrich. All NMR solvents were purchased from Cambridge Isotope Laboratories, Inc. and used without further purification. 2,2-bis(hydroxymethyl) propionic acid (Bis-HPA), Amberlyst® 15 Hydrogen Form (A-15), allyl glycidyl ether (AGE), anhydrous Iso-amyl alcohol (IAOH), 3,6-Dioxa-1,8-octanedithiol, Nile red, Coumarin 30, and 2,2-dimethoxy-2-phenylacetophenone (DMPA) were purchased from Sigma Aldrich and used without further purification. A sulfo-cyanine 3 carboxylic acid dye (Cy3 dye) was purchased from Lumiprobe Corporation and used without further purification. Glycidol (GLY) was purchased from Sigma Aldrich and vacuum distilled prior to use. Thiol PEG Thiol (HS-PEG-SH) (1,500 g/mol) was purchased from Nanocs and used without further purification. 2,2'-Azobis[2-(2-imidazolin-2-yl)propane]dihydrochloride (VA-044) was purchased from Wako chemicals and used without further purification. Tin(II) trifuoromethane sulfonate, $Sn(OTf)_2$, was purchased from Strem Chemicals Inc. and used without further purification. Teflon sheets were obtained from ePlastics. Dialysis membranes (Spectra/Por® 7, molecular weight cut-off (MWCO): 1,000 Da) were obtained from Spectrum Laboratories, Inc. 5-methyl-5-allyloxycarbonyl-1,3-dioxane-2-one (MAC), and 5-methyl-5-ethyloxycarbonyl-1,3-dioxane-2-one (MEC) were synthesized according to the literature and recrystallized prior to use.[1, 2] MEC/MAC copolymers were prepared according to our work previously published with molecular weights of 4000-4300 and PDI's of 1.[3]

### Characterization

$^1H$ NMR and $^{13}C$ NMR spectra were obtained from a Bruker 600 MHz Spectrometer, with $CDCl_3$/TMS or $MeOD_4$ as the solvent. Gel permeation chromatography (GPC) was carried out with a Waters chromatograph system equipped with a Waters 2414 refractive index detector, a Waters 2481 dual λ absorbance detector, a Waters 1525 binary HPLC pump, and four 5 mm Waters columns (300 mm x 7.7 mm), connected in series with increasing pore size (100, 1000, 100,000 and 1,000,000 Å respectively). All runs were performed with dimethylformamide (DMF) with LiBr (1 mg/mL) for the polyglycidol co-polymers using polyethylene glycol standards or tetrahydrofuran (THF) for the polycarbonates using polystyrene standards as the eluent at a flow rate of 1 mL/min.

### General Procedure for the synthesis of Poly (MEC MAC)

A 25 mL round bottom flask, equipped with stir bar, was capped with a rubber septum and flame dried under nitrogen. $Sn(OTf)_2$ (14mg; 33.6 µmol; 4 eq.) was then added to the round bottom flask and the reaction vessel was then immediately purged with nitrogen again prior to the addition of IAOH (37 mg; 2.42 mmol; 50 eq.) via microsyringe. The initiator-catalyst mixture was then allowed to stir at room temperature for 30 minutes before adding the MEC (1.58 g = 8.4 mmol, 1000 eq.) and MAC (420.2 mg = 2.1 mmol, 250 eq.). The reaction flask was then lowered into an 80 °C oil bath and the reaction was allowed to proceed until stirring was impeded. The resulting polymer product was dialyzed against DCM in tubing with a MWCO of 1 kDa for 3 days with 5 solvent changes. The pure polymer product was collected and dried on the rotavap to yield the

pure polymer product (1.60 g, 80% yield). The feed ratio of the monomers was 80/20, leading to the same incorporation in the final polymers. $^{1}$H NMR (400 MHz, $CDCl_3$/TMS, ppm) δ: 5.91-5.85 (m, -$OCH_2CHCH_2$), 5.34-5.23 (m, -$OCH_2CHCH_2$), 4.64-4.62 (m, -$OCH_2CHCH_2$) 4.40-4.15 (m, MAC and MEC, -$OC(O)OCH_2$), 1.30-1.22 (m, MAC and MEC, $CH_3$; MEC, -$OCH_2CH_3$), 0.93-0.91 (d, 3-methyl-1-butanol, $OCH_2CH_2CH(CH_3)_2$).

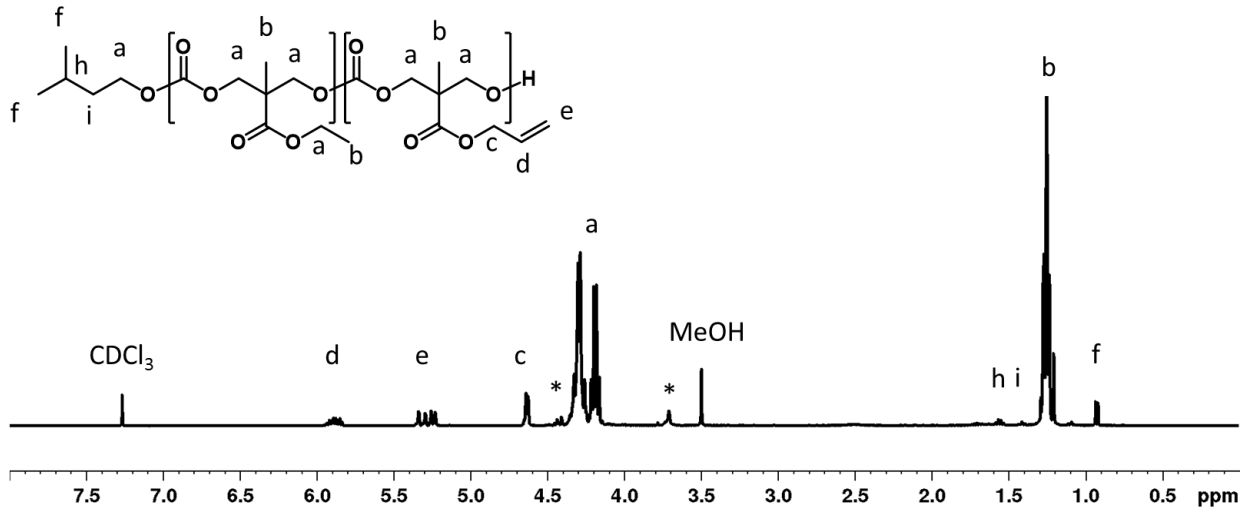



**Figure 1S.** $^{1}$H NMR in $CDCl_3$ of polycarbonate copolymer (* = protons from terminal unit on polymer).

**General Procedure for the synthesis of Poly (GLY AGE)**

A 25 mL round bottom flask, equipped with stir bar, was capped with a rubber septum and flame dried under nitrogen. $Sn(OTf)_2$ (5.2 mg; 12.48 µmol; 0.4 eq.) was then added to the round bottom flask and the reaction vessel was then immediately purged with nitrogen again prior to the addition of IAOH (43.7 mg 495.6 µmol; 17.4 eq.) via microsyringe. The initiator-catalyst mixture was then allowed to stir at room temperature for 30 minutes before lowering the reaction vessel into an ice water bath. After the reaction vessel had been cooled for 5 minutes the AGE monomer (834 mg; 7.31 mmol; 250 eq.) was added drop wise to the stirring reaction. The GLY monomer (2.17g; 29.24 mmol; 1000 eq.) was added drop wise in 4 separate aliquots, allowing 5 minute breaks between each aliquot, to ensure the exothermic reaction did not overheat. After stirring was completely impeded (~14 hours) the crude reaction mixture was solubilized in a minimal amount of methanol and precipitated into vigorously stirring ethyl acetate. The precipitate was allowed to settle before carefully decanting the ethyl acetate. The resulting GLY/AGE copolymer was solubilized in methanol, removed to a weighed 6-dram vial, and dried on vacuum to afford the translucent, viscous product (2.25 g= 75%). The feed ratio of the monomers was 75/25 leading to an 80/20 incorporation of the monomers in the final polymer. $^{1}$H-NMR (600MHz, MeOD) δ: 6.0-5.90 (m, –$OCH_2CHCH_2$), 5.37–5.17 (m, –$OCH_2CHCH_2$), 3.97-3.42 (6H). $^{13}$C-NMR (150MHz, MeOD) δ: 136.31, 117.39, 81.37, 79.81, 75.12, 73.88, 72.01-72.94, 70.42-71.17, 64.41, 62.53, 62.06, 20.85, 14.47. $M_w$ = 7480, $M_n$ = 4600, PDI = 1.60. Degree of branching: 0.21

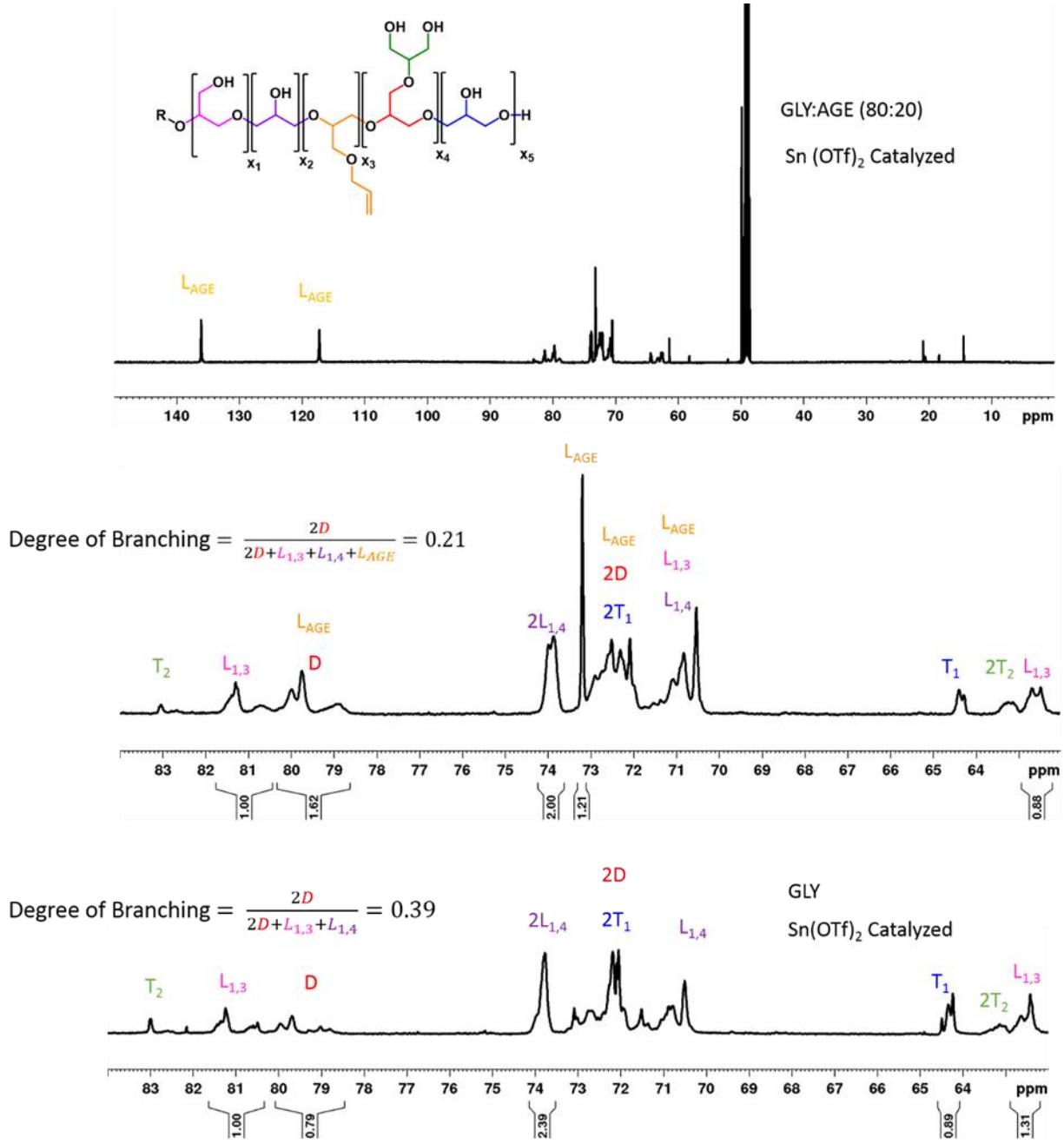



**Figure 2S.** Inverse gated $^{13}$ C NMR of the poly(GLY AGE), top. The middle spectrum shows a close up reaching from 83-62 ppm of the poly(GLY AGE) spectra. The bottom spectrum shows as comparison the homopolymer of polygycidol prepared with the same technique.

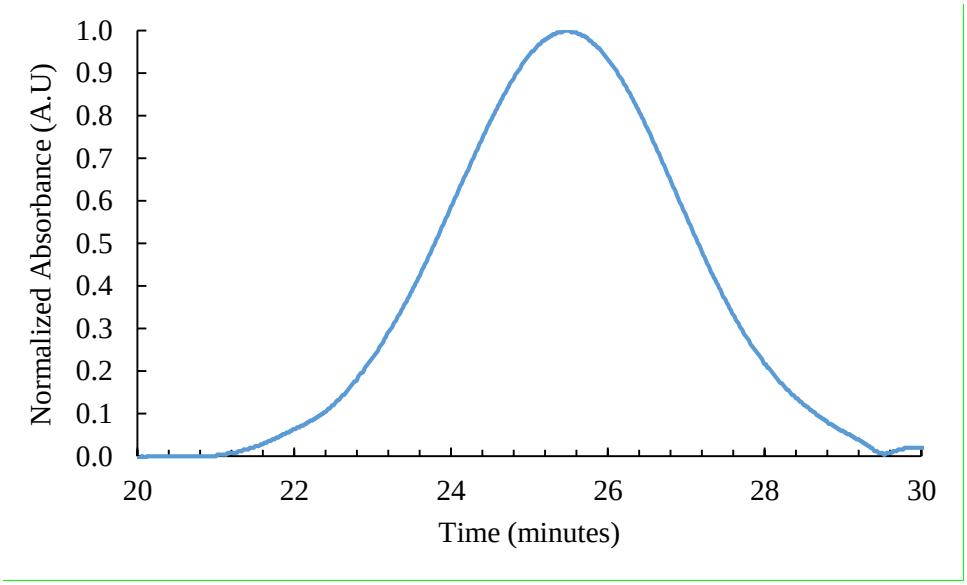



**Figure 3S.** GPC trace of poly(GLY AGE) with dimethylformamide (DMF) with LiBr as eluting solvent (1 mg/mL) using polyethylene glycol standards.

**Piezoelectric Inkjet Printing of Organic Ink for Microparticle Fabrication**

A Dimatix 2831 ink jet printer was used to print ink solutions of three different compositions.

In a general procedure for ink 1and ink 2, the allyl functionalized polymers, poly(MEC MAC) and poly(GLY AGE) were solubilized in DMSO before the addition of the DMPA initiator calculated for 0.2 eq per alkene. After manual mechanical mixing, the dithiol 3,6-Dioxa-1,8-octanedithiol (0.5 eq per alkene), was added via syringe to the solution and again mixed to form a homogenous solution.
The order of the addition for the various components was varied in the water-soluble ink 3. Here, we dissolved the poly(GLY AGE) and solid dithiol-PEG (1.5 KDa), 0.5 eq per alkene, in half of the desired amount of water until completely dissolved, followed by the second half of water containing the water soluble photoinitiator (VA-044).

Example with R.U. = 952.2 g/mol poly(MEC MAC) with 20% MAC unit and R.U. 410.5 g/mol poly(GLY AGE) with 20% AGE unit.
For the calculations, the exact percentile of incorporation of the allyl functionality has to be determined by NMR spectroscopy. If the molecular weight is unknown or cannot be exactly determined, the molecular weight of the repetition unit containing the allyl functionality (AGE) or (MAC), can be used to determine the quantity of the allyl groups of the sample to calculate the exact amount of the initiator and cross-linker used in the stated equivalents.

**Ink 1:** 9.57 mg (8.55µl) = 52.5 µmol of 3,6-Dioxa-1,8-octanedithiol (182.3 g/mol, 0.5eq. per alkene) via micro syringe to a solution of 100 mg poly(MEC MAC), 2,2-dimethoxy-2-phenylacetophenone (DMPA, 0.2 eq. per alkene, stock solution), and 1 mg of nile red in DMSO

in 200 µL. We also describe this ink as "50% m/v", which means a 1:1 ratio of mg/µL, and was optimized to a ratio of 1:2, "33% m/v".

**Ink 2:** 13.36 mg (11.94µl) = 73.3 µmol 3,6-Dioxa-1,8-octanedithiol (182.3 g/mol, 0.5eq. per alkene) was added via micro syringe to a solution of 70 mg poly(MEC MAC), 2,2-dimethoxy-2-phenylacetophenone (DMPA, 0.2 eq. per alkene, stock solution), 30 mg poly(GLY AGE) copolymer and 1 mg of Coumarin 30 in 400 µL DMSO. We also describe this ink as "25% m/v" which is a ratio of 1:4 mg/µl.

**Ink 3:** 182.7mg = 121.8µmol of HS-PEG-SH (1,500 g/mol, 0.5eq. per alkene) was dissolved in 0.5 ml distilled water together with 100 mg poly(GLY AGE). A second solution of 0.5 ml distilled water and 15.74 mg = 48.72 µmol 2,2'-Azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride (VA-044, 0.2eq. per alkene) and < 1 mg water-soluble sulfo-cyanine carboxylic acid Cy3 dye was then added to the first solution and mixed. We also describe this ink "10% m/v" which means a ratio of 1:10 mg/µl.

**Loading of the ink into cartridges, printing and imaging:**

After the ink was prepared, it was injected into a printer cartridge (Dimatix Materials Cartridge Model # DMC-11601/PN 700-10701-01) using a glass pipet. For both ink compositions using DMSO as the solvent we printed on glass coated with a sacrificial polyglycidol coating. These substrates were prepared using a spin coater from Laurell Technologies Corporation, Model WS-400A-6NPP/LITE. A solution of 1 g of polyglycidol in 1 g of methanol was used to spin-coat at 3500 rpm for 25 seconds. The polyglycidol was prepared as previously reported.[4] For the ink using water as a solvent, Teflon sheets as substrates were used. Once the printer cartridge and appropriate substrates were prepared the cartridge was inserted into the printer and the substrates were loaded onto the printer platform, respectively. The printer cartridge was then set to 37 °C and the recommended manufacturer's waveform was chosen at maximum voltage of 40 V. Lastly, the spacing between the printed droplets were set for placement of 100 microns apart from center to center in rows and columns on a 12" x 8" platform. After printing, the particles were exposed to long wave UV light to initiate the crosslinking.

A Nikon AZ 100M wide field microscope, equipped with a 5x Plan Fluor was used to image the microparticles that had been printed on the substrates with varying excitation wavelengths based upon the dye contained within each particle. Particles containing Nile Red were irradiated with 543 nm light, while 488 nm was utilized for the coumarin particles, and 633 nm for the sulfo-Cy3 species. The substrates were then washed with water in order to transfer the microparticle species into cell culture plates for imaging on a Zeiss LSM 510 inverted confocal microscope to determine the size of the free floating particles. While the same wavelengths were utilized, in this system two lens were used, which included a 10x Plan Neofluar lens for wide field imaging to determine particle uniformity, and a 63x Plan-APOCHROMAT OIL lens for investigation of single particles.

**Unconfined Compression Testing: Mechanical Studies**

The hydrogel samples for mechanical testing were prepared as previously described. The resulting hydrogel products were tested in triplicate at a rate of 1mm/min on an Instron 5944. The compressive modulus was determined using the initial linear region for each sample on the stress strain curve.

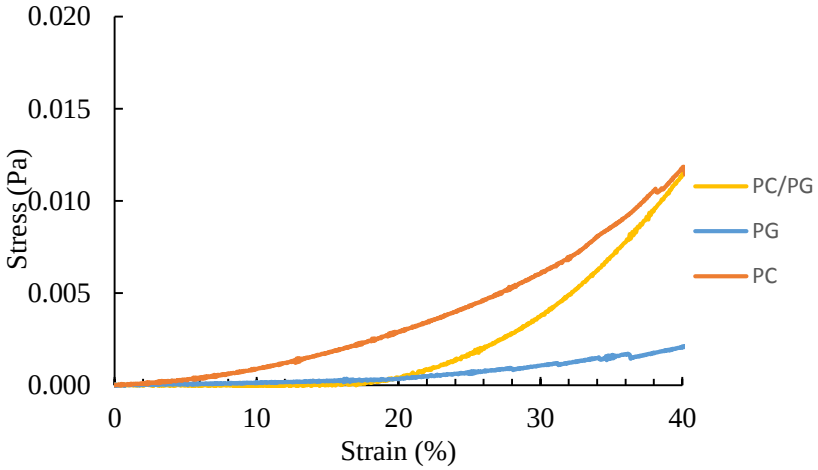



**Figure 4S.** Stress vs. strain curve of PC, PG, and PC/PG hydrogels.

The PG and PC/PG hydrogels have similar strength and elasticity up to 20%, which is demonstrated by the linearity of the stress-strain curve. At strains greater than 20% both the PG and PC/PG hydrogels become less elastic; however, the PG/PC gel has a higher strength. The PC is stronger than both the PG and PC/PG hydrogels and exhibits the least amount of elasticity.

**Table 1S.** Compositions of the four different inks investigated in this work.

| Ink (w/v) = (mg/µl) | MEC/MAC (mg) | AGE/GLY (mg) | Dithiol Cross-linker (MW) | Initiator | Solvent | Fluorescent Dye |
|---|---|---|---|---|---|---|
| 1 PC (1:2) | 100 | 0 | 182 g/mol | DMPA | DMSO (200µL) | Nile Red |
| 2 PC/PG (1:4) | 70 | 30 | 182 g/mol | DMPA | DMSO (400µL) | Coumarin 30 |
| 2a PC/PG (1:5) | 70 | 30 | 182 g/mol | DMPA | DMSO (500µL) | Coumarin 30 |
| 3 PG (1:10) | 0 | 100 | 1,500 g/mol | VA-044 | Water (1000µL) | Cy3 |

**Swelling Studies**

Bulk hydrogels of the compositions shown in Table 1S, were prepared for the swelling studies and were used to determine the suitability of the prepared ink for the printing process. Once the respective hydrogel was formed, the gels were then rinsed sequentially with 3mL aliquots of water, methanol, methylene chloride, methanol and finally water again to remove any unreacted starting materials. The hydrogels were frozen before being lyophilized in preparation for swelling studies. Prepared gels were soaked in deionized water and allowed to swell for 24 hours. The swelled gels were then gently blotted dry before recording the swelled mass ($M_{sw}$), and the sample was then lyophilized to record the dry mass ($M_{dry}$). The percent water content post-swelling was quantified using the following equation:

$$((M\_sw - M\_dry))/M\_dry \times 100\%$$

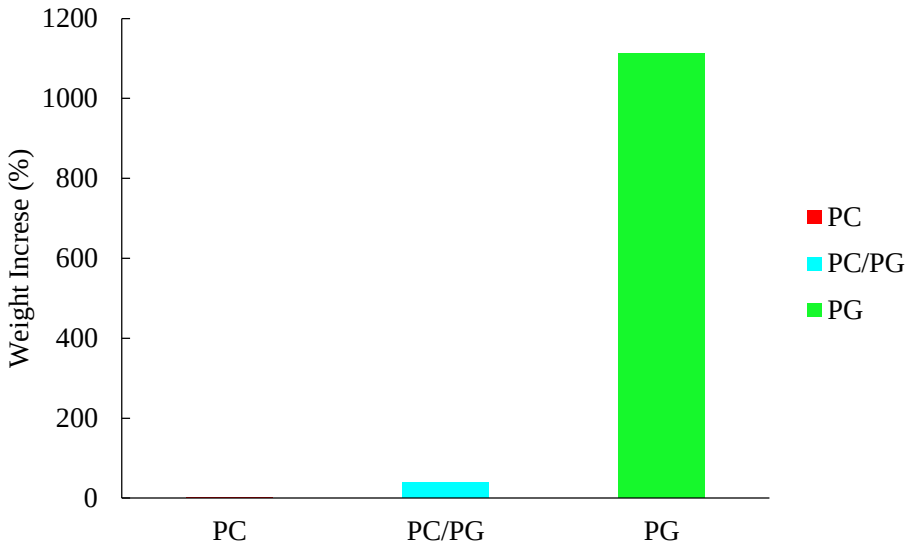



**Figure 5S.** Weight increase (%) of the PC, PC/PG, and PG lyophilized hydrogels after soaking in deionized water at 25 °C. The gels were allowed to soak for 24 hours before weighing.

The PC gel showed a 1 ± 1 % weight increase after soaking in water. The PC/PG gel showed a weight increase of 38 ± 6 %. Whereas the PG showed a weight increase of 1112 ± 236 %.

**Additional images of printed particles for better visualization:**

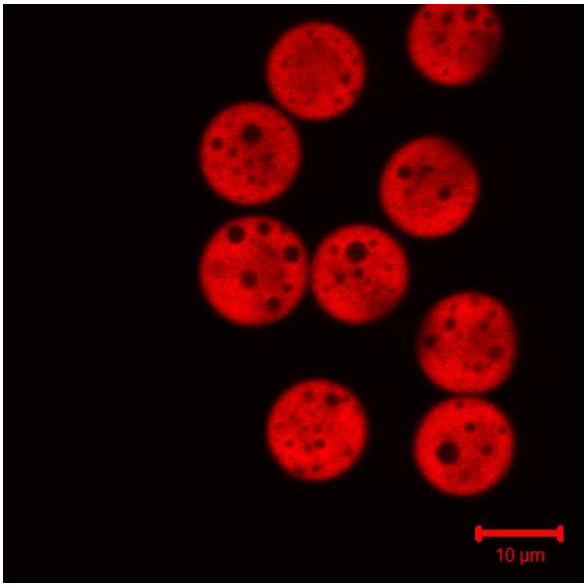
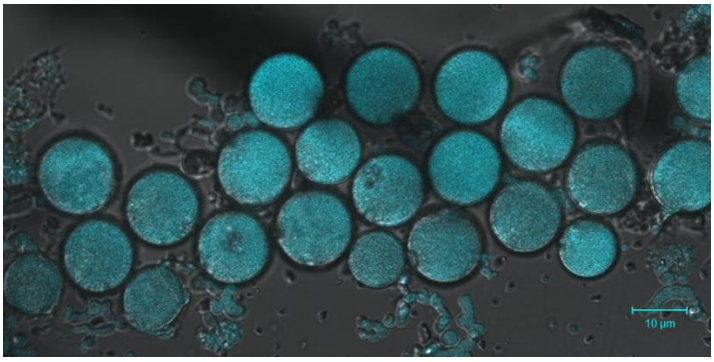



**Figure 6S**. Confocal image of polymer ink 1.



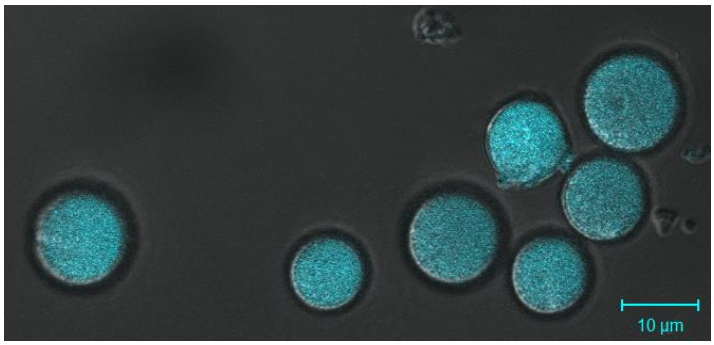




**Figure 7S and 8S**. Confocal image of polymer ink 2.

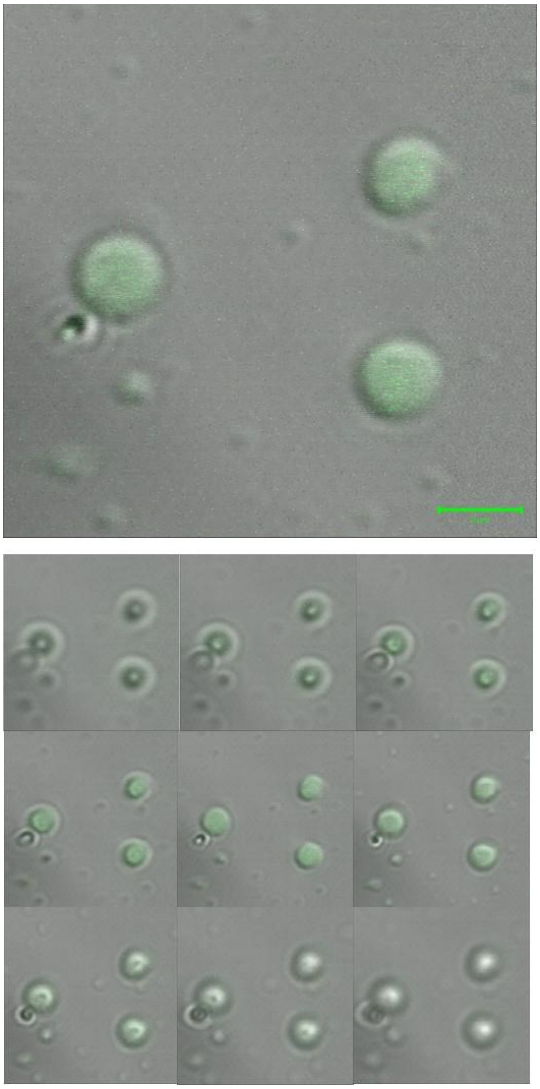


**Figure 9S**. Confocal image and Z-stack image of polymer ink 3.

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