Electronic Supplementary Material (ESI) for Soft Matter.

3D printed nitric oxide-releasing poly(acrylic acid)/F127/cellulose nanocrystal hydrogels

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Supporting Information

S-nitrosoglutathione (GSNO) characterization

GSNO synthesis was confirmed by UV-Vis spectrophotometry (HP-88453-Hewlett-Packard/Agilent) based on the detection of its characteristic absorption bands at $\lambda = 336$ nm ($n_O \rightarrow \pi^*$ electronic transition, **Fig S1A,B**) and $\lambda = 545$ nm ($n_N \rightarrow \pi^*$ electronic transition, **Fig S1C,D**). Spectra of freshly prepared aqueous solutions with concentrations in the range of 0.2 to 0.7 mM (for the band at 335 nm, **Fig S1A**) and 18 – 28 mM (for the band at 545 nm, **Fig S1C**), were measured in quartz cuvettes (1 cm optical path). The molar absorption coefficients, calculated from the corresponding absorbance vs. concentration plots (**Figs. S1B** and **S1D**) are: $\varepsilon_{335} = 720\ M^{-1}\ cm^{-1}$ and $\varepsilon_{545} = 11\ M^{-1}\ cm^{-1}$, in accordance with values described elsewhere.[1]

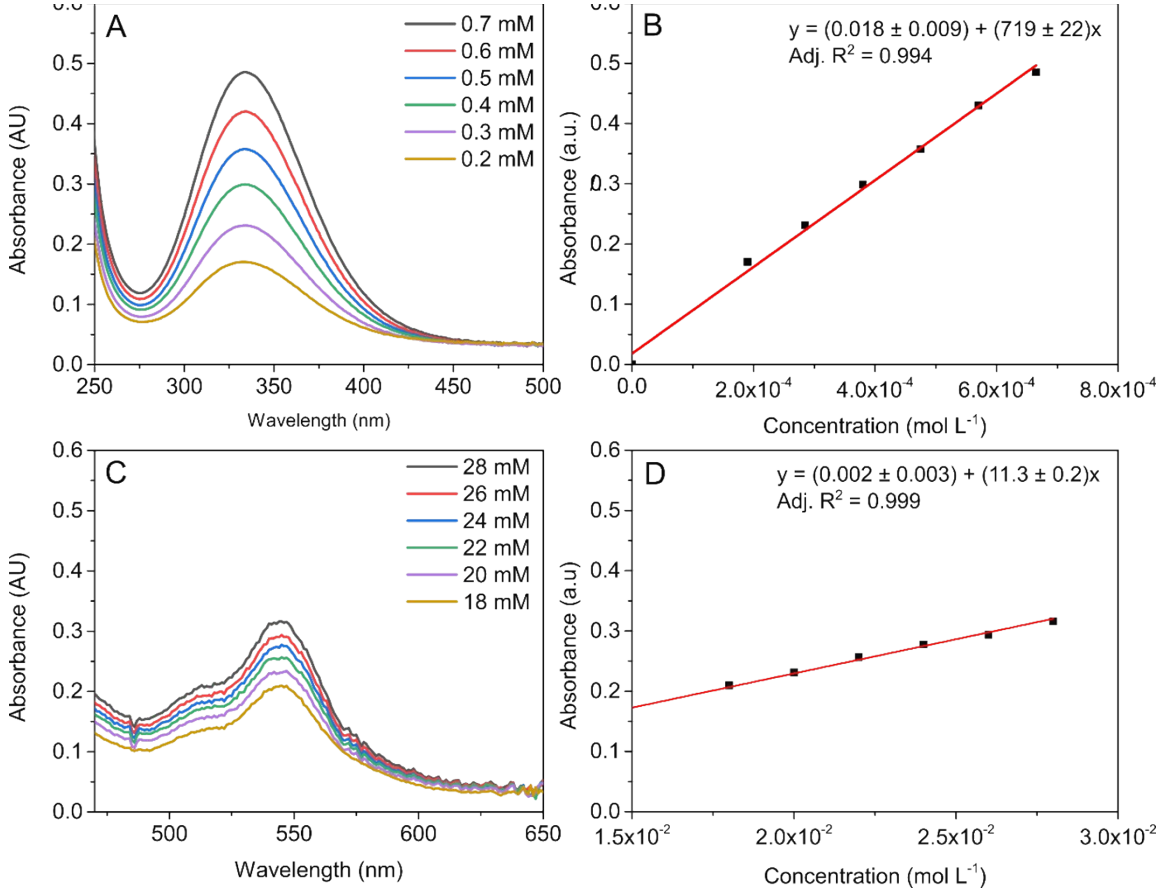

Fig S1. UV-Vis spectra of aqueous GSNO solutions. (A) Absorption spectra showing the absorption band with maximum at $\lambda = 335$ nm for several concentrations in the 0.2 to 0.7 mM range. (B) Plot of absorbance at 335 nm vs. concentration. (C) Absorption spectra showing the absorption band with maximum at $\lambda = 545$ nm for several concentrations in the 18 to 28 mM range. (D) Plot of absorbance at 545 nm vs. concentration.

GSNO quantification by UHPLC

GSNO chromatographic purity was measured by Ultra High Performance Liquid Chromatography (UHPLC) in a liquid chromatographer (Acquity, Waters, Millford – MA, USA) using reduced L-glutathione, GSH (≥ 98.0%) and oxidized L-glutathione, GSSG (≥ 98.0%), (Sigma Aldrich) as standards. GSNO was analyzed in the 100 – 400 µM concentration range. Samples of 10 µl were injected into a Luna C18(2) column (particle size - 5 µm, pore size – 100 Å, 4,6 mm x 250 mm, Phenomenex, Torrance – CA, USA) at ambient temperature. The mobile phase consisted of a 90:10 (v/v) mixture of a 0.1% trifluoroacetic acid (TFA) aqueous solution (pH 2.2) and a 40:60 (v/v) acetonitrile:water solution pumped at 1.0 ml min^{-1} flow rate. The elution was monitored for 6 min using UV detection at 220 nm. Representative chromatograms obtained for 400 µM GSH, GSSG and GSNO solutions are shown in **Fig. S2**.

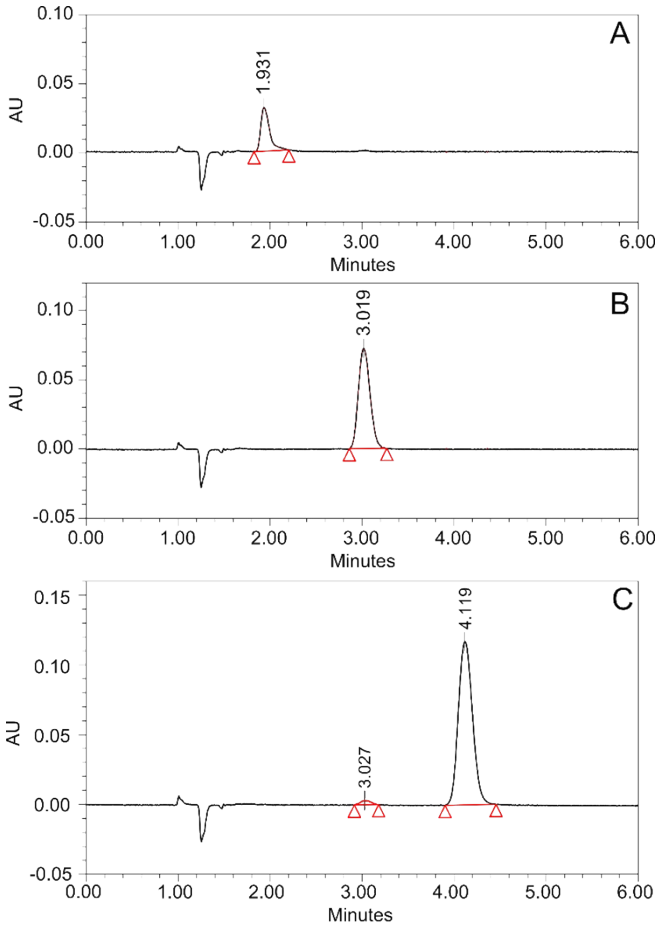

Fig S2. Representative chromatograms of (A) reduced L-gluthatione, GSH, (B) oxidized L-glutathione, GSSG and (C) GSNO.

The measured retention times were 1.9 min, 3 min and 4.1 min for GSH (**Fig. S2A**), GSSG (**Fig. S2B**) and GSNO (**Fig. S2C**), respectively. While no trace of remaining GSH was detected in the GSNO chromatogram, a small peak assigned to GSSG was present, indicating that the GSNO solution underwent some decomposition. This analysis allowed estimating a chromatographic purity of 95.3 % for GSNO (**Table S1**).

Table S1. Chromatographic calibration parameters of GSH and GSSG and chromatographic purity of GSNO estimated from the percentages of peak areas.

	Slope	Intercept	Adj R-Square	**Peak area (%)**
GSH	$5.2523\ 10^{13} \pm 5.7825\ 10^{11}$	$-2.100\ 10^{3} \pm 1.551\ 10^{3}$	0.9997	**n.d.**
GSSG	$1.5799\ 10^{14} \pm 1.5929\ 10^{12}$	$1.6005\ 10^{3} \pm 4.232\ 10^{3}$	0.9998	**4.7 ± 1.2**
GSNO	-	-	-	**95.3 ± 1.2**

Stability of GSNO solutions

The extent of thermal decomposition of the 5 mM, 10 mM and 20 mM GSNO solutions used for the charging of the PAA/F127/GSNO and PAA/F127/GSNO hydrogels was characterized spectrophotometrically based on the monitoring the decay of the UV-Vis characteristic absorption bands of GSNO over the 24 h period of the post-printing charging process. Spectra were taken immediately after the preparation of the solutions and after 4, 8 and 24 h, while keeping the solutions at 25 °C, protected from room light.

The spectral changes recorded at these four time points are shown in **Fig. S3.** The UV band at 335 nm was used for the 5 mM GSNO solution, using a quartz cuvette with 0.1 cm optical path, while the band at 545 nm was used for the 20 mM GSNO solution, using a quartz cuvette with 1 cm optical path. The extents of decomposition, calculated from the absorption changes using the molar absorption coefficients described in **Fig. S1,** were: 9.2 ± 0.1 %, 28.3 ± 0.4 % and 36.5 ± 0.1 % for the 5 mM, 10 mM and 20 mM GSNO solutions, respectively, after 24 h.

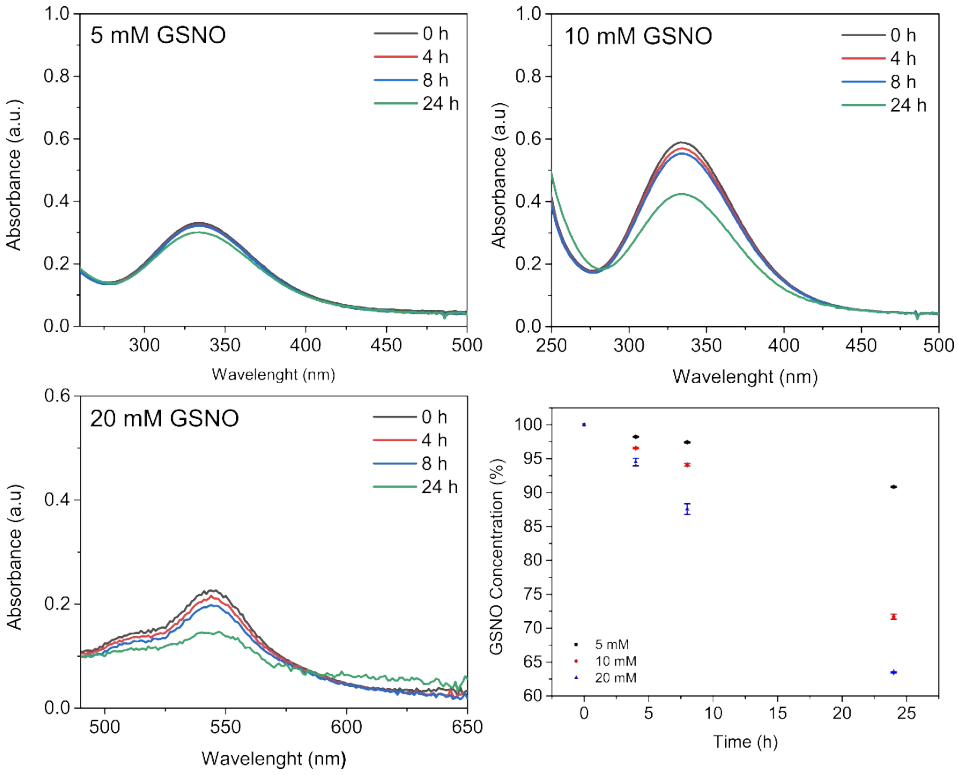

Fig S3. Spectral changes of GSNO solutions 5, 10 and 20 mM after 4, 8 and 24 h kept at 25 C, protected from room light, and the corresponding extents of decomposition, calculated from the changes in the absorptions at 335 nm (for concentrations 5 and 10 mM) and 545 nm (for concentration 20 mM).

GSNO charge measurements

The GSNO charges in PAA/F127 and PAA/F127/CNC hydrogels were measured spectrophotometrically. Dried PAA/F127 and PAA/F127/CNC0.25 disks were weighed and immersed in 5 mM, 10 mM and 20 mM GSNO solutions during 24 h. The GSNO absorbance was measured at 335 nm for 5 mM and 10 mM solutions and at 545 nm for 20 mM solutions, immediately prior to the hydrogel's immersion (time zero) and immediately after the removal of the hydrogels (24 h). The molar amounts of GSNO absorbed from the solutions based on the absorbance changes according to the Beer-Lambert law, using molar absorption coefficients experimentally determined (ε_{335} ~ 720 L mol^{-1} cm^{-1}, ε_{545} ~ 11 L mol^{-1} cm^{-1}), taking into consideration the percentages of GSNO decomposed (**Fig S3**). The results are shown in **Table S2**. All measurements were performed in triplicate and are expressed as mean ± standard deviation.

Table S2. Nominal and measured GSNO concentrations in the solutions used to charge the PAA/F12/ and PAA/CNC0.25 hydrogels though absorption from solution and GSNO charges estimated from absorption changes after 24 h of charging.

GSNO concentration (mM)		GSNO charge (μmol g^{-1})	
Nominal	Measured*	PAA/F127/GSNO	PAA/F127/CNC0.25/GSNO
5	4.5 ± 0.1	0.8 ± 0.3	0.60 ± 0.02
10	8.3 ± 0.1	4.0 ± 0.1	3.8 ± 0.5
20	19.5 ± 0.1	6.0 ± 0.1	11.9 ± 1

*Calculated from the absorbances measured at 335 nm (5 and 10 mM) and 545 nm (20 mM).

Vibrational spectral analysis

Fourier Transformed Infrared spectra (FTIR) of dried hydrogels and resin components (AA, MBA, F127, CNCs) were obtained by depositing the samples directly over the ZnSe crystal of a Cary 630 FTIR spectrophotometer (Agilent, Santa Clara – CA, USA) operating in the 4000-400 cm^{-1} range and ATR mode. The FTIR spectra are depicted in **Fig. S4**.

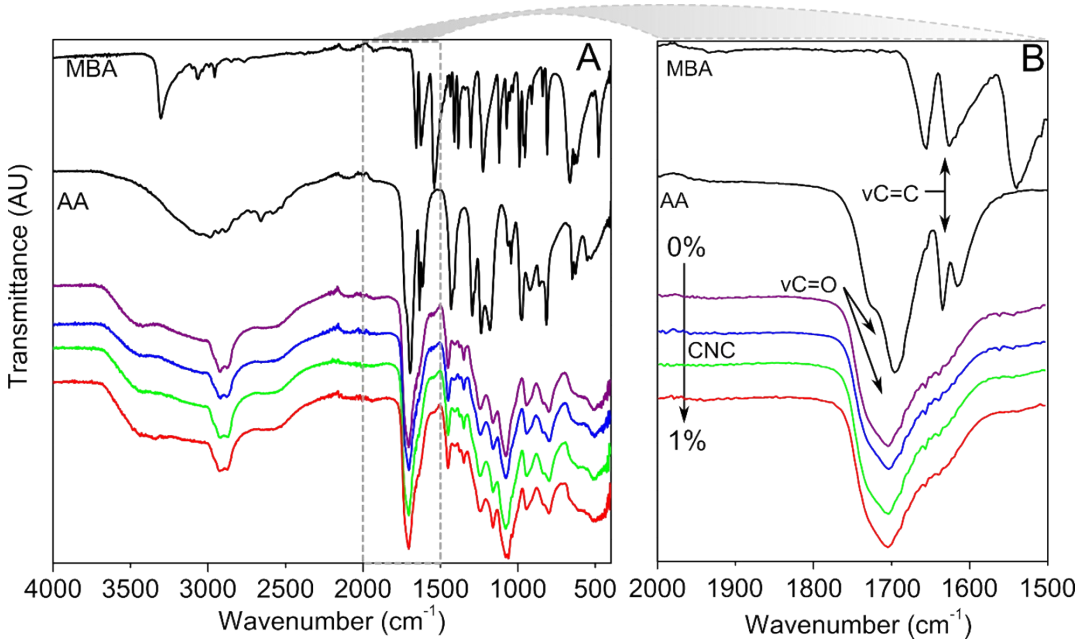

Fig S4. (A) FTIR spectra of pure N,N'-methylenebisacrylamide, MBA, pure acrylic acid, AA, and four different PAA/F127/CNC hydrogels with increasing cellulose nanocrystal, CNC, concentration, from top to bottom (indicated by the different colors), in the 4000 cm^{-1} – 400 cm^{-1} wavenumber range. (B) Magnification of the 2000 cm^{-1} – 1500 cm^{-1} wavenumber range, showing the vanishing of the absorption band assigned to the vinyl groups of AA during the formation of PAA.

3D printed constructs

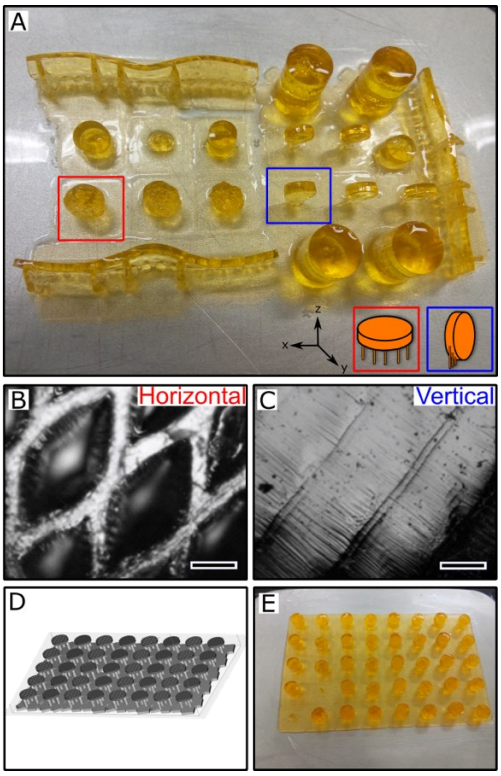

Fig S5. Representative 3D printed constructs printed in different orientations: (A) Photograph of the printing table where horizontally and vertically oriented 3D printed PAA/F127 hydrogel honeycomb discs are indicated in red and blue, respectively. (B) and (C) are the corresponding optical micrographs. (D) representative 3D model used in the printing of (E). Scale bars in (B) and (C) represent 500 µm.

Transmission Electron Micrographs

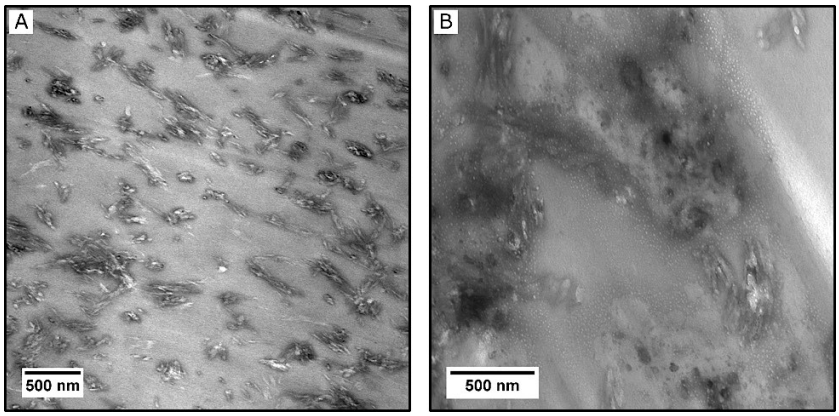

Fig S6. Representative TEM micrographs showing CNC dispersion in PAA/F127/CNC1 hydrogels containing (A) 7.5 wt% and (B) 20 wt% of F127.

X-ray diffraction analysis of PAA/F127/GSNO hydrogels

X-ray diffraction (XRD) was used to assess GSNO dispersion in a vacuum-dried PAA/F127 hydrogel loaded with the highest GSNO concentration used in this study (20 mM). The diffractograms were obtained in the 8°–50° 2θ range, using a XRD 7000 diffractometer (Shimadzu, Kyoto, JPN) operating with Cu Kα-radiation wavelength (λ= 1.54 Å) at 40 kV voltage, 30 mA current and a 2° min^{-1} rate (**Fig S7).** While the diffractogram of pure GSNO exhibits several sharp Bragg peaks, which are characteristic of highly crystalline solids the diffractogram of the PAA/F127/GSNO shows only an amorphous halo in the same 2θ range, indicating the absence of GSNO crystals in the hydrogel in accordance with previous results reported elsewhere.[2]

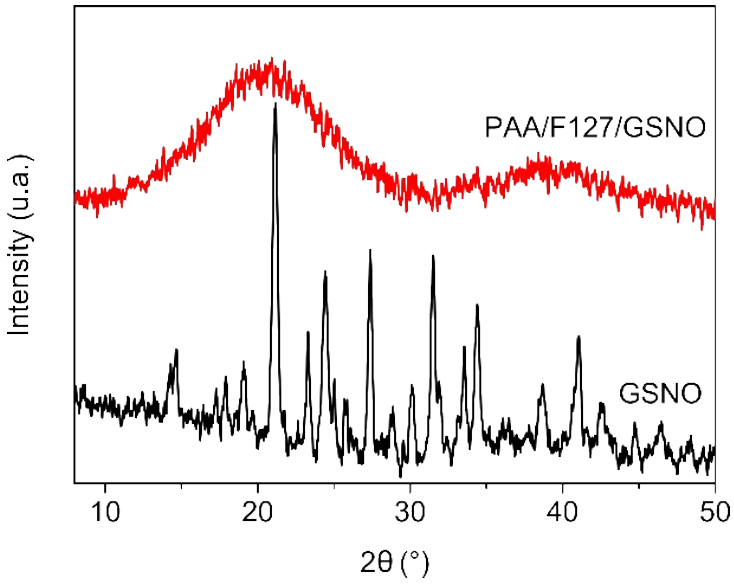

Fig S7. XRD patterns for pure GSNO and a PAA/F127/GSNO hydrogel charged with a 20 mM GSNO solution.

References

1 P. D. Wood, B. Mutus and R. W. Redmond, Photochem. Photobiol., 1996, **64**, 518–524.

2 M. Champeau, V. Póvoa, L. Militão, F. M. Cabrini, G. F. Picheth, F. Meneau, C. P. Jara, E. P. de Araujo and M. G. de Oliveira, Acta Biomater., 2018, **74**, 312–325.