Supporting Information

Scanning Electron Microscopy (SEM) Evaluation of Hydrogel Nanoparticles

Standard SEM imaging of hydrogel particles provides the nanoparticle length and width dimensions in the dried state. SEM images of 33% (v) PEGDA particles still attached to the imprinting substrate and released particles were taken and analyzed using Image J software to obtain the particle dimensions (length, width). From the images and AFMs scans of the particles, the heights of the particles are $\sim 120-140$ nm. TEM images of 50% (v) PEGDA hydrogel nanoparticles verify that the length and width values obtained from the 2-dimensional SEM and TEM images are depending on the particles random orientation on the substrate. Analysis of the 2-dimensional images shows two distinct populations of width values: one ~ 110 nm and the other ~ 140 nm. Based on the particles height values obtained from AFM, the height dimension of the particles was assumed to be the dimension that was measured to be over 115 nm in the twodimensional SEM images. Table S1 summarizes the average length, width, and area values obtained from SEMs of the released particles (n = 25 particles) in the assigned orientations. When the particle's orientation becomes a variable, the standard deviation of the length, width, and area values increases. The percent difference between the particle designed area, the dimensions that were patterned onto the S-FIL template, and the measured area obtained from the images also increases in comparison to the values obtained from the particles still attached to the substrate.

To obtain the nanoparticles dimensions in the non-dried state, QuantmiX wet SEM capsules were used. Using these capsules, nanoparticles can be imaged while in **Table S1: Nanoparticle dimension values obtained from SEM from released nanoparticles.** Length, width, and area values obtained from released nanoparticles (n of 25 particles). The area percent difference was calculated by comparing the theoretical area of each particle, based on the dimension patterned on the quartz template, versus the area measured from the SEMs images.

Particle Orientation 1					
	LENGTH (nm)	WIDTH (nm)	AREA (nm²)	AREA % DIFFERENCE	
811nm Rectangles	830 ± 40	146 ± 20	121141 ± 17000	60	
411nm Rectangles	350 ± 21	115 ± 33	40178 ± 12000	0.7	

Particle Orientation 2					
	LENGTH 2 (nm)	WIDTH 2 (nm)	AREA 2 (nm²)	AREA % DIFFERENCE	
811nm Rectangles	876 ± 19	204 ± 42	178418 ± 37000	69	
411nm Rectangles	375 ± 27	183 ± 64	68702 ± 24000	36	

aqueous solution and protected from the electron beam. **Table S2** provides the average and standard deviation of lengths, widths, and areas of 33% (v) and 50% (v), 811 nm and 411 nm PEGDA nanoparticles. Even with the particles electrostatically attached to the wet capsule membrane, the particle orientation is still random, making accurate length and width measurements difficult. Overall, the length and width values of the 50% (v) PEGDA 700 swollen nanoparticles was smaller then the 33% (v) PEGDA 700 nanoparticles. A significant increase in the nanoparticles length and widths were measured from the swollen nanoparticles (**Table S1**) in comparison to the length and width values measured from the dried released particles (**Table S2**) as shown in **Figure S1**. Table S2: Nanoparticles dimensions in swollen state using QuantmiX wet capsules Length, width, and area values obtained from released nanoparticles (n of 10 particles). The area percent difference was calculated by comparing the theoretical area of each particle, based on the dimension patterned on the quartz template, versus the area measured from the SEMs images.

33% (v) Hydrogel Particles Orientation 1				
	LENGTH (nm)	WIDTH (nm)	AREA (nm²)	AREA % DIFFERENCE
811nm Rectangles	969.58 ± 26.75	195.83 ± 11.35	189876.74 ±12000	81.43
411nm Rectangles	427.70 ± 31.63	161.10 ± 24.63	68901.93 ± 12000	53.08

33% (v) Hydrogel Particle Orientation 2				
	LENGTH 2 (nm)	WIDTH 2 (nm)	AREA 2 (nm²)	AREA % DIFFERENCE
811nm Rectangles	982.71 ± 30.91	232.79 ± 21.18	228761.85 ± 22000	96.36
411nm Rectangles	460.41 ± 43.21	228.83 ± 17.93	105357.41 ± 13000	89.93

50% (v) Hydrogel Particles Orientation 1

	LENGTH (nm)	WIDTH (nm)	AREA (nm²)	AREA % DIFFERENCE
811nm Rectangles	871.40 ± 22.49	121.35 ± 8.68	105744.39 ± 8100	27.72
411nm Rectangles	374.50 ± 7.40	121.35 ± 3.47	45445.575 ± 1600	12.75

50% (v) Hydrogel Particle Orientation 2				
	LENGTH 2 (nm)	WIDTH 2 (nm)	AREA 2 (nm²)	AREA % DIFFERENCE
811nm Rectangles	959.75 ± 76.12	164.77 ± 19.85	158139.87 ± 23000	65.63
411nm Rectangles	388.30 ± 24.24	142.00 ± 1.67	55138.6 ±3500	31.824



Figure S1: Comparison of swollen and dried particle dimensions measured from SEM. (A) 400 nm by 100 nm by 100 nm particle dimensions. (B) 800 nm by 100 nm by 100 nm particle dimensions. Data is presented as mean \pm mean standard error of n = 30 for 411 nm particles and n = 10 for 811 nm particles. **P* <0.001 indicates significant differences swollen and dried samples, determined with student t-test.

Atomic Force Microscopy (AFM) Evaluation of Hydrogel Nanoparticles

Utilizing AFM, 33% (v) and 50% (v) PEGDA 700 hydrogel nanoparticles, particles topographies were investigated after fabrication (relaxed state), dried state, and swollen state. **Figure S2** presents the particle dimensions in each state for the 50% (v/v) PEGDA (MW700) polymer hydrogels.



Figure S2: 50% (v) PEGDA 700 hydrogel nanoparticle topography values obtained using AFM. Data is presented as mean \pm total uncertainty for a sample size n = 60. Data with matching letters represents values that are different outside the range of measurement uncertainty.

Effect of surface tension

A combination of thermodynamics and rubber elasticity theories states that a crosslinked polymer gels that is immersed in a fluid swells under two opposing forces: the thermodynamic force of mixing and the retractive forces induced by the crosslinked polymer network. The swelling reaches equilibrium when these two forces are balanced. The total Gibbs free energy of the gel is

$$\Delta G_{total} = \Delta G_{mix} + \Delta G_{elastic} \tag{1}$$

where ΔG_{mix} is the free energy of mixing and $\Delta G_{elastic}$ is the increase of the free energy due to elastic stretch of the polymer network inside the gel. The free energy of mixing includes contributions from entropy (ΔS_{mix}) and enthalpy (heat, ΔH_{mix}), namely

$$\Delta G_{mix} = \Delta H_{mix} - T\Delta S_{mix} = k_B T [n_1 \ln \varphi + \chi n_1 (1 - \varphi)]$$
⁽²⁾

where n_1 is the number of solvent molecules, φ is the volume fraction of the solvent ($\varphi = 1 - v_{2,s}$), *T* is the absolute temperature, k_B is the Boltzmann constant, and χ is a dimensionless parameter that is specific to the gel and characterizes the interaction between the solvent and the polymer. The swelling of the gel is driven by the gain in entropy ($\Delta S_{mix} > 0$) of the system by the mixing of the solvent and polymer. The volume fraction of the solvent ($\varphi = 1 - v_{2,s}$) is related to the number of solvent molecules (n_1) in the gel:

$$\varphi = 1 - v_{2,s} = \frac{n_1 V_1}{n_1 V_1 + N_a V_{polymer}}$$
(3)

where V_1 is the molar volume of the solvent (e.g., water) and N_a is the Avogadro's number.

Based on the statistical theory of rubber elasticity [Flory, Treloar], the elastic free energy density was obtained as

$$\Delta G_{elastic} = \frac{n_e k_B T}{2} \left[\alpha_1^2 + \alpha_2^2 + \alpha_3^2 - 3 - \kappa \ln(\alpha_1 \alpha_2 \alpha_3) \right], \tag{4}$$

where α_1 , α_2 , and α_3 are degrees of stretches in three principal directions, n_e is the effective number of polymer chains in the network, and κ is a dimensionless parameter that equals 1 in the original theory but equals 2 in some modified theories [Hong et al, Kang and Huang]. When the hydrogel is unconstrained, it swells isotropically, with $\alpha_1 = \alpha_2 = \alpha_3 = \alpha$, which can be related to the polymer volume fraction as

$$\alpha^{3} = \frac{v_{2,r}}{v_{2,s}},$$
(5)

where $v_{2,r}$ is the polymer volume fraction at the relaxed state (reference configuration), defined as the state of the gel immediately after crosslinking but before swelling [Peppas and Merill].

Relative to the pure solvent, the chemical potential of the solvent in the swollen gel is

$$\mu_1 = \mu_{mix} + \mu_{elastic} \tag{6}$$

where

$$\mu_{mix} = \left(\frac{\partial}{\partial n_1} \Delta G_{mix}\right)_T = k_B T \left[\ln \left(1 - v_{2,s}\right) + v_{2,s} + \chi v_{2,s}^2 \right]$$
(7)

$$\mu_{elastic} = \left(\frac{\partial}{\partial\alpha}\Delta G_{elastic}\right)_T \left(\frac{\partial\alpha}{\partial n_1}\right)_T = \frac{n_e V_1 k_B T}{N_a V_{polymer}} \left(v_{2,r}^{2/3} v_{2,s}^{1/3} - \frac{\kappa}{2} v_{2,s}\right)$$
(8)

The effective number of polymer chains is related to the number average molecular weights as

$$n_e = \frac{N_a V_{polymer}}{\overline{v} \overline{M}_c} \left(1 - \frac{2 \overline{M}_c}{\overline{M}_n} \right), \tag{9}$$

where $\bar{\upsilon}$ is the specific volume of the polymer, \bar{M}_c is the number-average molecular weight between the cross-links, and \bar{M}_n is the molecular weight of the linear polymer chains before cross-linking. Therefore, Eq. (8) can be re-written as

$$\mu_{elastic} = \frac{k_B T V_1}{\overline{\nu} \overline{M}_c} \left(1 - \frac{2\overline{M}_c}{\overline{M}_n} \right) \left(v_{2,r}^{2/3} v_{2,s}^{1/3} - \frac{\kappa}{2} v_{2,s} \right)$$
(10)

When the gel reaches equilibrium, the chemical potential inside the gel equals that of the pure solvent and thus the relative chemical potential is zero. Combining Eqs. (7) and (10), one obtains an expression for determining \bar{M}_c in a neutral hydrogel:

$$\frac{1}{\overline{M}_{c}} = \frac{2}{\overline{M}_{n}} - \frac{\overline{\nu}}{V_{1}} \frac{\ln(1 - \nu_{2,s}) + \nu_{2,s} + \chi \nu_{2,s}^{2}}{\left(\nu_{2,r}^{2/3} \nu_{2,s}^{1/3} - \frac{\kappa}{2} \nu_{2,s}\right)}$$
(11)

This equation can then be used to determine \tilde{M}_c from the measurements of equilibrium swelling ratio (*Q*) or the polymer volume fraction (*v*_{2,s}).

For nanoscalehydrogel particles, a size effect is predicted theoretically by considering the effect of surface tension. In addition to the bulk free energy, the surface free energy of the hydrogel is:

$$\Delta G_{surface} = S\gamma, \tag{12}$$

where γ is the surface energy density (or surface tension) and *S* is the surface area of the hydrogel particle. As the hydrogel swells, the surface area increases. For isotropic, homogeneous swelling, the surface area is

$$S = \alpha^2 S_0 = S_0 \left(\frac{v_{2,r}}{v_{2,s}}\right)^{2/3},$$
(13)

where S_0 is the surface area in the relaxed state. Correspondingly, the surface-induced chemical potential is

$$\mu_{surface} = \left(\frac{\partial}{\partial \alpha} \Delta G_{surface}\right)_T \left(\frac{\partial \alpha}{\partial n_1}\right)_T = \frac{2S_0 V_1 \gamma}{3N_a V_{polymer}} v_{2,r}^{2/3} v_{2,s}^{1/3}$$
(14)

where the surface tension has been assumed to be a constant during swelling. Combining Eq. (14) with Eqs. (7) and (10), the total chemical potential of the solvent in the hydrogel is

$$\mu_1 = \mu_{mix} + \mu_{elastic} + \mu_{surface} \tag{15}$$

By setting $\mu_1 = 0$ for the equilibrium state, we obtain that

$$\left[\ln(1-v_{2,s})+v_{2,s}+\chi v_{2,s}^{2}+\frac{V_{1}}{\overline{\nu}\overline{M}_{c}}\left(1-\frac{2\overline{M}_{c}}{\overline{M}_{n}}\right)\left(v_{2,r}^{2/3}v_{2,s}^{1/3}-\frac{\kappa}{2}v_{2,s}\right)\right]+\frac{2V_{1}\gamma}{3RT}\frac{S_{0}}{V_{0}}\left(\frac{v_{2,s}}{v_{2,r}}\right)^{1/3}=0,$$
(16)

where V_0 is the volume of the hydrogel at the relaxed state (thus $V_{polymer} = V_0 v_{2,r}$), and *R* is the molar gas constant.