ADVANCED HEALTHCARE MATERIALS

Supporting Information

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Crystalline Antibody-Laden Alginate Particles: A Platform for Enabling High Concentration Subcutaneous Delivery of Antibodies

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Supplementary Information for "Crystalline Antibody-Laden Alginate Particles: A Platform for Enabling High Concentration Subcutaneous Delivery of Antibodies"

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1. Process Design Consideration for the Centrifugal Extrusion Process

Effect of sodium alginate type on hydrogel particles is illustrated in Figure S1. Effect of surfactant on particle shape is illustrated in Figure S2.

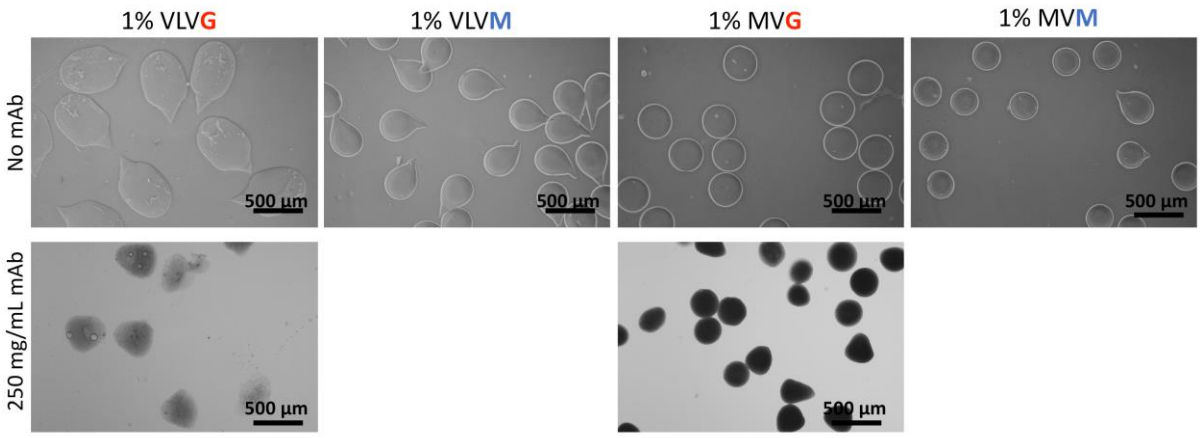

Figure S1. Alginate hydrogel particles formed with guluronic (G) rich and mannuronic (M) rich alginate. VLVG and VLVM solution apparent viscosity are < 20 mPa·s with molecular weight of < 75 KDa. MVG and MVM apparent viscosities are > 200 mPa·s with molecular weights of > 200 kDa. Low viscosity (low molecular weight) alginate is more prone to deformation. Alginate hydrogels formed at: 2mm dispenser-to-bath, 200 RCF, 50 mM $CaCl_2$, 30G dispenser, no surfactant.

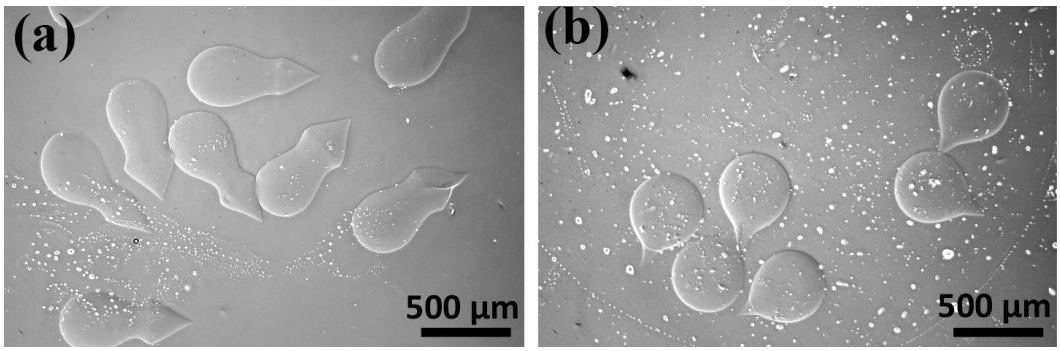

Figure S2. Effect of Tween 20 noon-ionic surfactant on 1% VLVG alginate particle shape. **(a)** No Tween 20. **(b)** 0.02 % v/v Tween 20. Particles formed at 50 mM $CaCl_2$, 200 RCF, 0 mg/mL antibody, 30 G dispenser and 2 mm dispenser-to-bath distance.

The effective volume fraction of the antibody laden alginate particles. To measure this, particles were centrifuged at 3000 RCF for 10 minutes and the excess PEG 10% w/v solution was removed (from the top) using a micropipette. The effective volume fraction (ϕ) was

measured gravimetrically by measuring the mass of hydrogel particles and suspension separately:

$$\phi = \frac{V_p}{V_{susp.}} = \frac{\rho_p m_p}{\rho_{susp.} m_{susp.}} \qquad \text{Eq. S1}$$

in which V_p and $V_{susp.}$, ρ_p and ρ_{susp} , and m_p and m_{susp}, are the volume fraction, density, and mass of the particles and the suspension.

In Figure 2a. The centrifugal acceleration caused the fluid to flow downwards. As a result, droplets of the pre-gel were formed at the tip of the dispenser. The dimensionless numbers that govern this "dripping regime" are the Oh, We, and Bo numbers:[1]

$$Oh = \frac{\mu}{\sqrt{\rho R \gamma}} \qquad \text{Eq. S2}$$

$$We = \frac{\rho U^2 R}{\gamma} \qquad \text{Eq. S3}$$

$$Bo = \frac{\rho a R^2}{\gamma} \qquad \text{Eq. S4}$$

in which R, ρ, and a are the radius of the dispenser, suspension density and centrifugal acceleration, respectively, while μ and γ, are the pre-gel viscosity and surface tension. Also, U is the cross-sectionally averaged velocity of the pre-gel at the nozzle outlet.

The throughput (given by the volumetric flow rate Q) of this encapsulation method is dictated by the flow rate of the pre-gel in the dispenser which can be approximated based on the Hagen–Poiseuille equation:

$$Q = UA = \frac{\pi R^4 \rho a H}{8 \mu L} \qquad \text{Eq. S5}$$

in which H and L are total height of the liquid and the dispenser length respectively. The device throughput is limited by the high flow boundaries that allow a "dripping regime" (instead of fluid jet).[1] The estimated flow rate can be up to 5 ml/hr product (at 300 mg/ml particle loading) from a single dispenser apparatus (calculated for R= 0.08 mm, ρ=1100 kg/m^3, a= 1000 RCF , H= 6.27 cm, μ= 0.6 Pa.s (measured), γ= 0.05 N/m,[2] and L= 1.27 cm). The estimated throughput is comparable to high-throughput microfluidic devices and the scale-up can be achieved by simultaneous operation of multiple dispensers and/or devices. Furthermore, the calculated values for the dripping regime dimensionless numbers are *Oh*=9.0, *We*=0.13, and *Bo*=5.19.

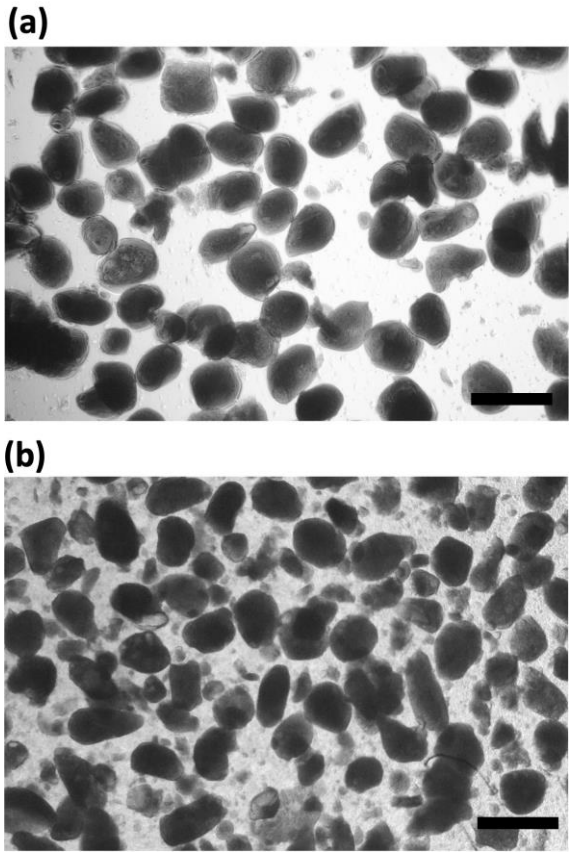

Figure S3. Crystalline mAb-laden particles before *(a)* and after *(b)* ejection from the 27-gauge (ID=210 μm) needle, commonly used in SC administration. Particles at mAb loading of 250 mg/ml. Scale bars are 400 μm.

2. Forming Hydrogel Particles Using Partially Oxidized Alginates

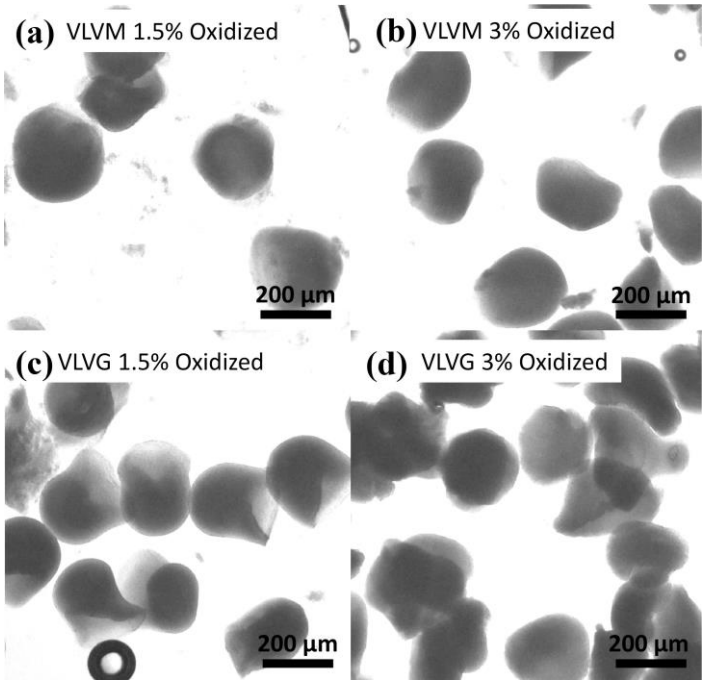

Figure S4. Forming mAb laden alginate particles using partially oxidized alginates. Our results indicated that both mannuronic rich and guluronic rich alginates of up to 3% uronic oxidized were able to form hydrogel particles.

Hydrogel swelling is a result of liquid absorption by the polymeric network and is defined as:

$$Swelling\ ratio = \frac{m_h - m_d}{m_d} \qquad \text{Eq. S6}$$

in which m_h and m_d are the mass of the hydrated (swollen) and dried hydrogel respectively. The "initial" measurement refers to hydrogels after the crosslinking in the bath while the equilibrated refers to hydrogels immersed in SBF for 24 hours. Firstly, for the non-modified alginates, ALG M has a lower swollen ratio than the ALG G which explains its slower slightly slower initial degradation. The modified alginates have higher swollen ratios.

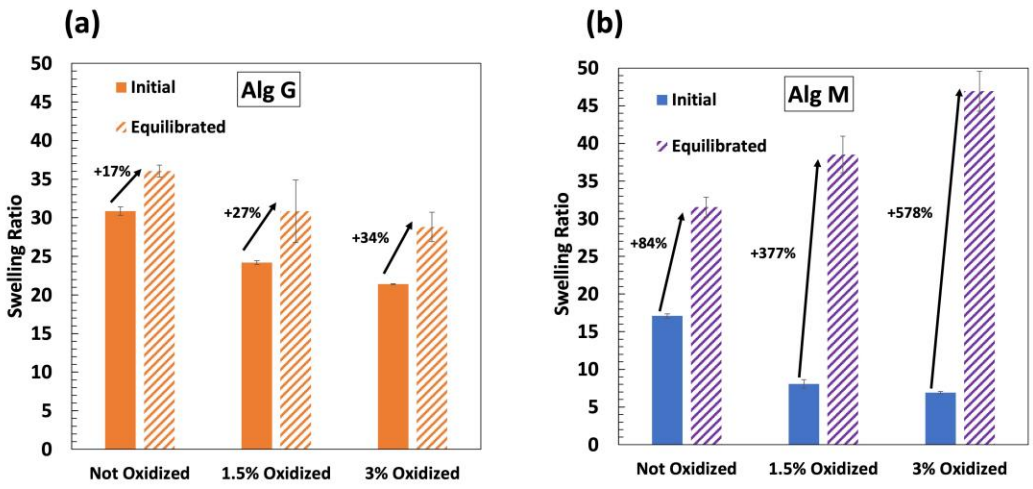

Figure S5. Swelling ratio of the hydrogel particles correlates with the disintegration rate of the hydrogels. **(a)**: Guluronic rich alginate (ALG G): G/M >1.5. **(b)**: mannuronic rich alginate (ALG M): G/M <1.

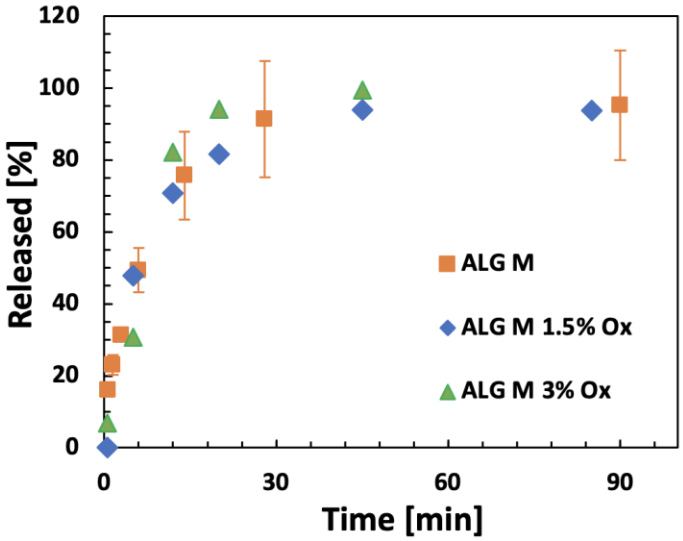

Figure S6. Dissolution and release of the anti-PD-1 antibody from the alginate hydrogel particles in simulated body fluid (SBF) at 37 °C. The release of mAb from partially oxidized alginates followed the similar trend with the unmodified alginates. Particles at 150 mg/ml mAb particle loading.

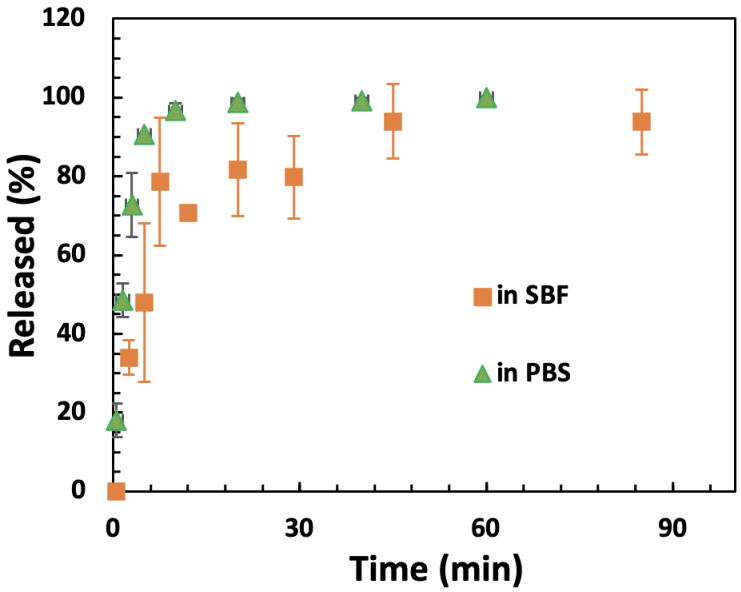

Figure S7. Release of the anti-PD-1 antibody from the alginate hydrogel particles in phosphate buffered saline (PBS) followed the similar fast and complete release trend observed for the release in simulated body fluid (SBF) at 37 °C. Particles at 200 mg/ml mAb particle loading.

3. Quality of the Released Antibody

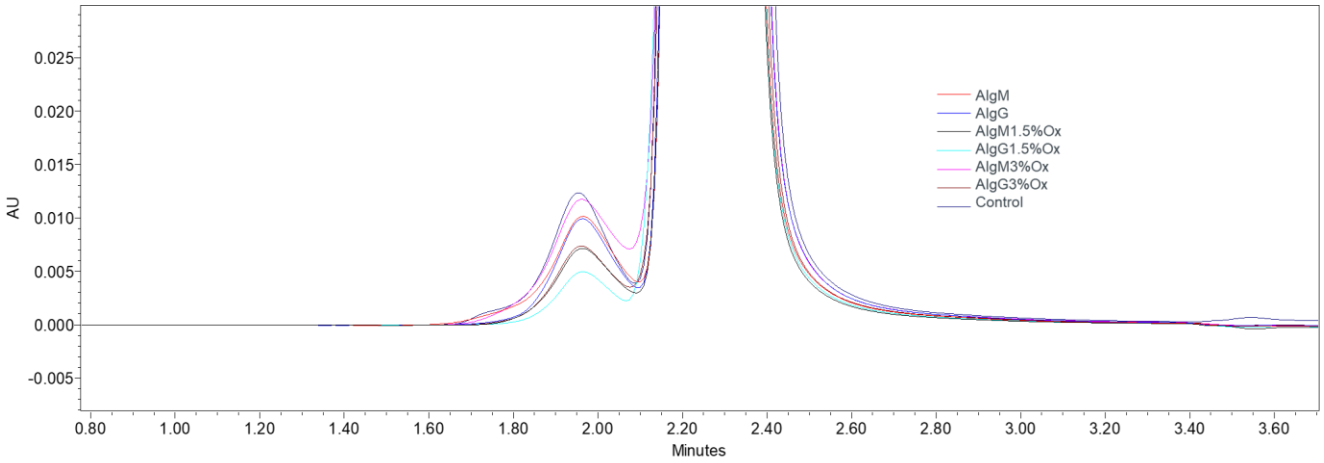

Figure S8. Size exclusion chromatography (SEC) for the mAb released from different alginate particles. The SEC data indicates that the entire process has not resulted in any perceivable increase in the number of aggregates and is an indicative of the compatibility of the proposes process with the monoclonal antibody.

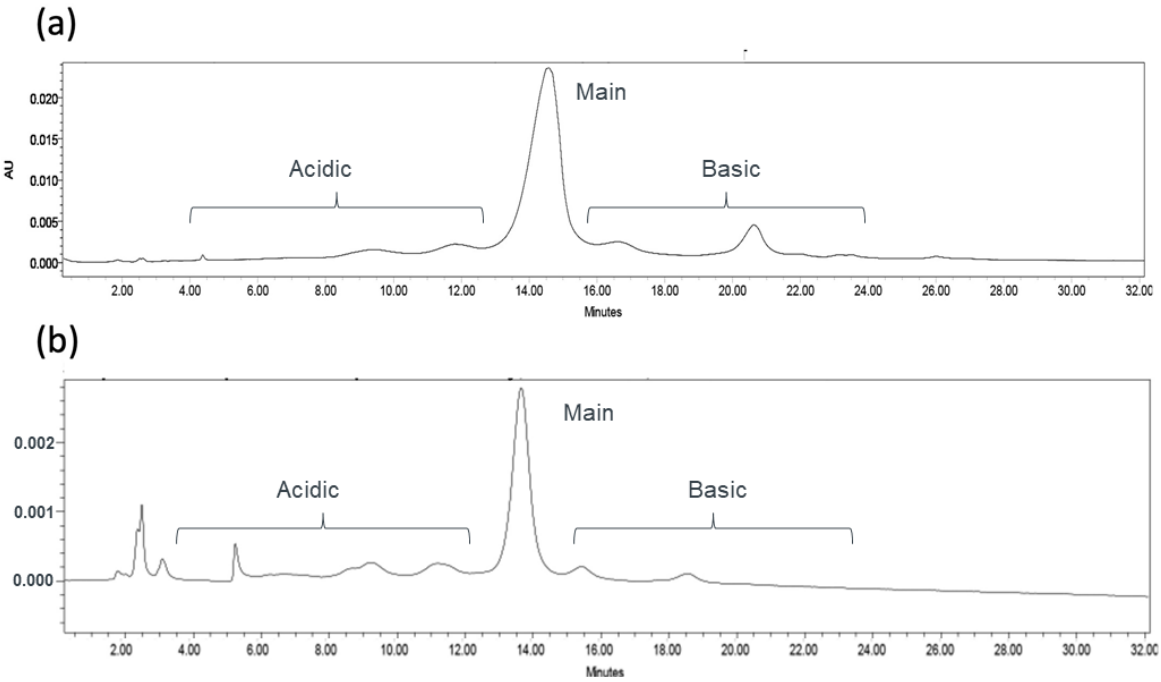

Figure S9. Ion exchange (IEX) chromatographs of (a) the standard (control) monoclonal antibody and (b) the monoclonal antibody encapsulated in the hydrogel and later released. The control antibody (a) and the antibody released from the alginate hydrogel particles (b) showed profiles of charge variants with similar amounts of the major acidic and basic peaks. Some additional peaks eluting between 2.00-4.00 min for the released antibody sample (b), likely represent the materials from the alginate hydrogel.

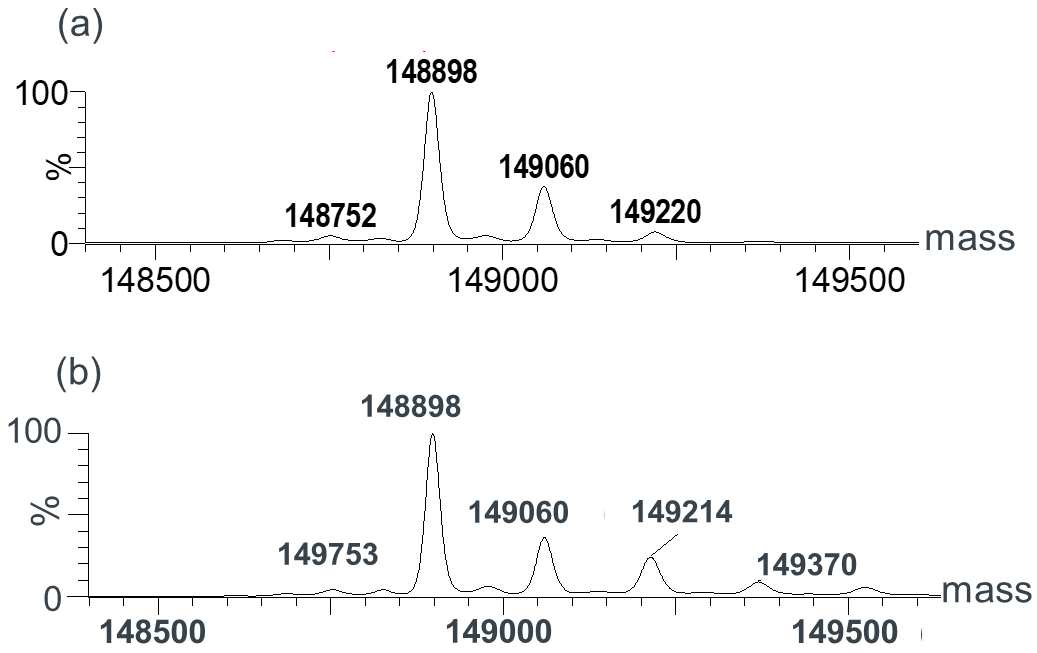

Figure S10. Liquid chromatography mass spectrometry (LC–MS) of (a) the standard (control) monoclonal antibody and (b) the monoclonal antibody encapsulated in the hydrogel and later released.

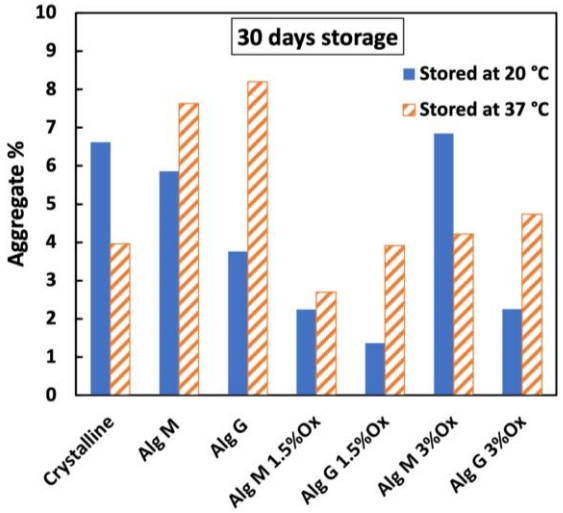

Figure S11. Total % of high molecular weight soluble aggregates for the crystalline anti-PD-1 antibody formulations (at 100 mg/ml particle loading) stored at room and elevated temperatures.

We formed the crystalline mAb laden hydrogel particles using two other chemistries and methods. For the Case 1, free radical reaction was carried out using poly(ethylene glycol) diacrylate (PEGDA, molecular weight 700 Da) and 2-hydroxy-2- methylpropiophenon as the photoinitiator.[3] For the Case 2, hydrogel particles were prepared by Michael-type addition thiol-vinylsulfone click-chemistry using batch emulsion reaction. For this purpose, 4-arm polyethylene glycol vinylsulfone (PEGVS, MW 10 KDa) and PEG-dithiol (PEGDT, MW 3.4 KDa) were used. Hydrogel particles were prepared in a 10 mL stirred-batch oil emulsions using mineral oil ("light mineral oil", M3516). Equimolar mixture (based on endgroups) of PEGVS (4 endgroups), and PEGDT (2 endgroups) were prepared at 10% w/v total PEGVS/PEGDT in 50 mM HEPES buffer, 10% w/v PEG 3350 and the antibody crystals at pH 8. This prepolymer suspension was dripped into the mineral oil with 0.3 v/v Span 80 and stirred at 200 rpm for 2 hours for the particles to form.

4. In Vitro

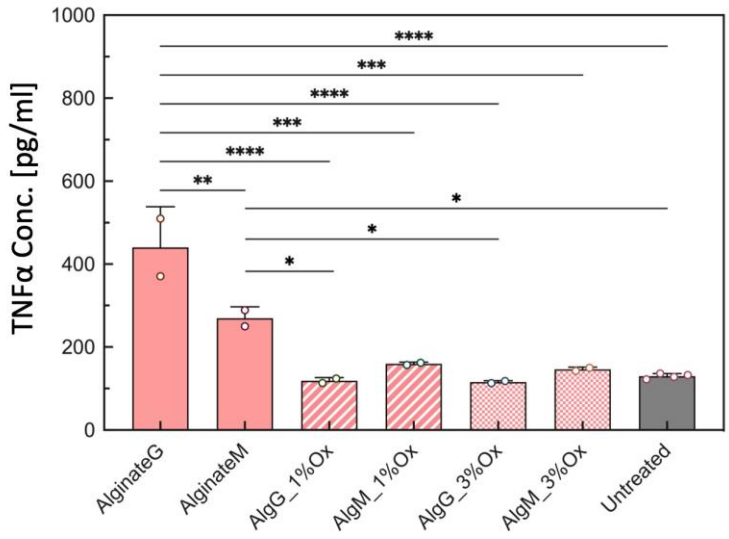

Figure S12. Non modified alginates hydrogel particles cause a small yet statistically significant increase in the secretion of TNFα. Interestingly the partially oxidized alginates particles do not result in secretion of TNFα.

Table S1. Endotoxin levels measured in the samples.

Sample	Endotoxin level [EU/ml]
Caffeine solution	0.011
mAb-ALG G	0.052
mAb-ALG M	0.046
mAb-ALG G 3% Ox	0.026
mAb-ALG M 3% Ox	0.034

5. Supplemental Videos

Video S1: Manual ejection of hydrogel suspension prepared at 100 mg/ml anti-PD-1 antibody using a 27G needle.

Video S2: Manual ejection of hydrogel suspension prepared at 200 mg/ml anti-PD-1 antibody using a 27G needle.

References

1. Eral, H. B.; Safai, E. R.; Keshavarz, B.; Kim, J. J.; Lee, J.; Doyle, P., Governing principles of alginate microparticle synthesis with centrifugal forces. *Langmuir* **2016,** *32* (28), 7198-7209.
2. Kamaruddin, M. A.; Yusoff, M. S.; Aziz, H. A., Preparation and characterization of alginate beads by drop weight. *International Journal of technology* **2014,** *5* (2), 121-132.
3. Schieferstein, J. M.; Reichert, P.; Narasimhan, C. N.; Yang, X.; Doyle, P. S., Hydrogel Microsphere Encapsulation Enhances the Flow Properties of Monoclonal Antibody Crystal Formulations. *Advanced Therapeutics* **2021,** *4* (4), 2000216.