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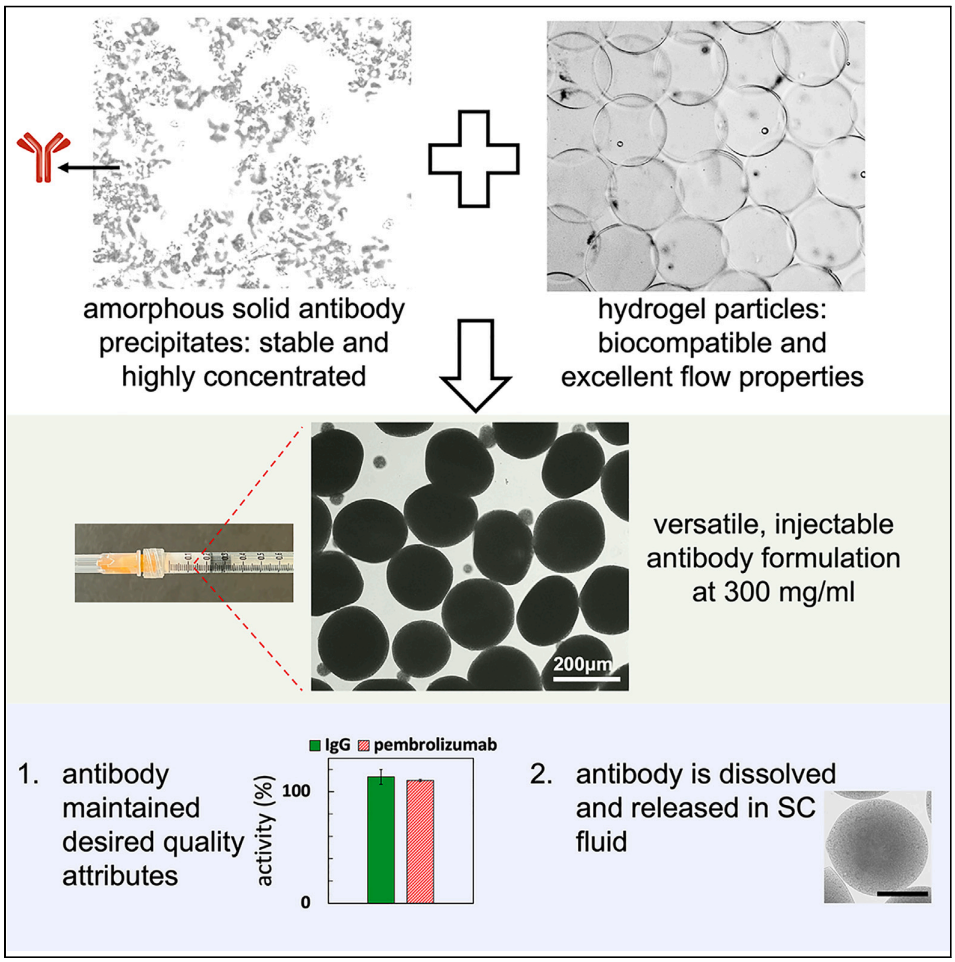

Amir Erfani, Paul Reichert, Chakravarthy N. Narasimhan, Patrick S. Doyle

pdoyle@mit.edu

Highlights

A generalizable injectables dosage form for high-concentration antibody formulations

Eliminating the need for drying to reach high concentrations

Amorphous antibody precipitates were encapsulated in alginate hydrogel particles

The soft hydrogel matrix allowed packing the particles to high volume fractions

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Injectable hydrogel particles for amorphous solid formulation of biologics

Amir Erfani,[1] Paul Reichert,[2] Chakravarthy N. Narasimhan,[2] and Patrick S. Doyle[1,3,4,*]

SUMMARY

The fast pace of breakthroughs in cancer immunotherapy, combined with the new paradigm of moving toward high-concentration dosages and combinatorial treatments, is generating new challenges in the formulation of biologics. To address these challenges, we describe a method of formulation that enables high-concentration injectable and stable formulation of biologics as amorphous solids in aqueous suspension. This technology combines the benefits of liquid formulation with the stability of solid formulation and eliminates the need for drying and reconstitution. This widely applicable formulation integrates the amorphous solid forms of antibodies with the injectability, lubricity, and tunability of soft alginate hydrogel particles using a minimal process. The platform was evaluated for anti-PD-1 antibody pembrolizumab and human immunoglobulin G at concentrations up to 300 mg/mL with confirmed quality after release. The soft nature of the hydrogel matrix allowed packing the particles to high volume fractions.

INTRODUCTION

During the last decade there have been several breakthroughs in the treatment of cancer[1] and auto-immune disease[2] through administration of biologics, notably monoclonal antibodies (mAbs). For each new biologic drug, a significant challenge encountered by the manufacturers is to develop cost-effective formulations that are stable and can be administrated with ease (e.g., injectable). Yet there are paradigm changes in new proposed treatments that can challenge the current state of the art in formulation of biologics. Ever increasing number of therapies in clinical trials and intense competition require drug forms that can be developed relatively fast. Furthermore, because of the high dosing level requirements, and increased interest for subcutaneous (SC) administration, mAbs are desired at increasingly high concentrations.[3–6]

There are several challenges in formulating antibodies as either liquid or freeze-dried solids. Liquid mAbs at high concentrations become increasingly viscous and more prone to aggregation, unfolding, and degradation.[5,7] In contrast, solid forms of antibodies do not pose colloidal stability problems, and the improved stability results in a longer shelf life. Currently, solid formulation products are formed by freeze-drying, which is expensive, and the process parameters are research intensive and need optimization for each new mAb. Furthermore, solid forms of mAbs are formulated to be reconstituted just prior to injection which poses a significant inconvenience to the end user.[8,9] Additionally, all commercially available solid formulations of mAbs are reconstituted at low concentrations of $\leq$ 100 mg/mL (due to point-of-care limitations), thus limiting the dosing quantity at acceptable injection volumes.

A multifaceted solution to these challenges can be achieved by designing a dosage form that has the stability benefits of a solid formulation, combined with the convenience of a liquid formulation. Simultaneously, this dosage form should allow high concentrations (for SC administration) while being economically feasible to produce and adaptable to industrial-scale continuous production.

Amorphous solid forms of mAbs have the benefit of being highly stable compared to liquid formulations.[8] Amorphous solid mAbs can be produced by variety of methods[10] including polyethylene glycol (PEG)-induced precipitation.[11] These non-crystalline native aggregates can be produced by reversible precipitation with almost complete yields. Suspensions of amorphous solid proteins can be concentrated by

[1]Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA 02142, USA

[2]Merck & Co., Inc., Kenilworth, NJ 07033, USA

[3]Harvard Medical School Initiative for RNA Medicine, Boston, MA 02215, USA

[4]Lead contact

*Correspondence: pdoyle@mit.edu

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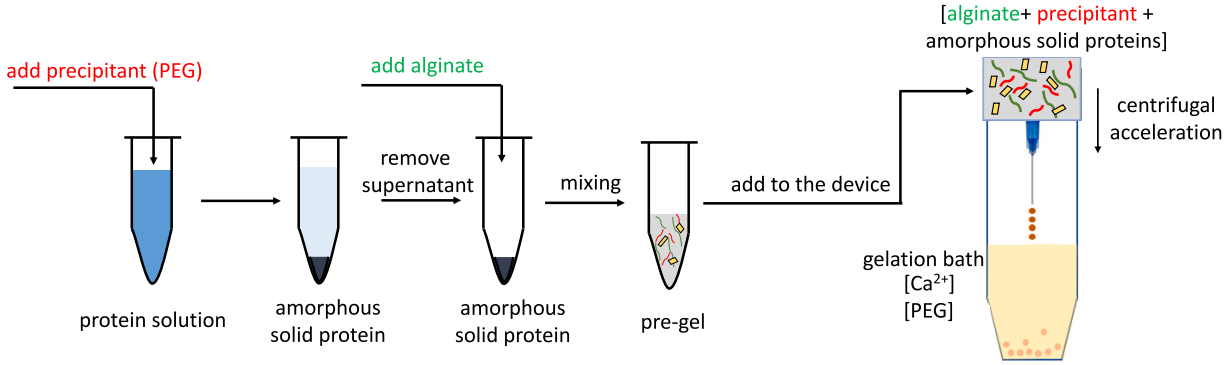

Figure 1. Schematic of the encapsulation process

Using polyethylene glycol (PEG) as a precipitant, amorphous solid antibodies were formed. Next, the solid antibodies were mixed with sodium alginate to form the pre-gel suspension. The suspension was then extruded through a dispenser using centrifugal force to form droplets. The pre-gel droplets formed a gel upon impact with the gelation bath containing $CaCl_2$ and PEG.

centrifugation, sedimentation, or filtration and can dissolve outside of the precipitation conditions. Formulating mAbs as amorphous solids can be a desired formulation solution if injectability can be facilitated.

Hydrogels (cross-linked networks of hydrophilic polymers) are viable candidates for SC or local delivery of mAbs.[12–20] Importantly, hydrogel particles are soft and lubricious resulting in favorable shear-thinning flow properties even at high volume fractions.[21–23] Sodium alginate is a natural polysaccharide that can form a hydrogel by ionic cross-linking in mild conditions without use of adversely toxic reactants, making it widely used for biomedical applications.[24–29] Alginate has a track record of safe clinical use for a wide variety of biomedical applications.[30,31]

In this work we propose a minimal, generalizable, simple-to-develop, and scalable process to form injectable solid formulation of biologics. We integrate the stability of amorphous solid proteins with the desired flow properties of hydrogel microspheres to form a novel dosage form for biologics. The work presented here builds upon our previous work on crystalline forms of antibodies[32] by expanding it to amorphous precipitates forms of antibodies that can be generally formed with relative ease. We demonstrate the generalizable nature of the process by encapsulating both an mAb and a polyclonal antibody as model therapeutics.

RESULTS

Encapsulation of amorphous solid antibody in alginate particles

As illustrated in Figure 1, first, solid forms of antibody were prepared via precipitation. Next, the solid particles were mixed with a sodium alginate solution, and subsequently amorphous solid antibody-laden alginate hydrogel particles were formed using a simple microfluidic device.[33] Amorphous solid antibodies were formed by precipitation of the antibody in 16.6% w/v PEG 3350 kDa solution (precipitant). This process led to almost complete antibody recovery (99.5% w/w $\pm$ 0.1). The antibody precipitates were then resuspended in 2% w/v sodium alginate solution and concentrated using centrifugation to form the pre-gel suspension. The pre-gel suspension was filled inside the device which consists of a dispenser (nozzle) and a $CaCl_2$ bath for cross-linking. The dispenser used was a blunt-tip, 30-gauge needle (outer diameter: 0.32 mm, inner diameter: 0.16 mm, length: 12.70 mm) with threaded Luer lock taper. Subsequently, using centrifugal acceleration, the pre-gel suspension was extruded through the dispenser to form droplets that cross-linked upon impact with the $CaCl_2$ bath.[33] Both the pre-gel suspension and the $CaCl_2$ bath contained 10% w/v PEG to avoid dissolution of the amorphous solid antibody by steric exclusion of antibody from the aqueous phase.[11] In this minimal process, only two gentle steps (precipitation and encapsulation) were required for preparing the dosage form.

Figure 2 illustrates the amorphous solid immunoglobulin G (IgG) and pembrolizumab and their encapsulated forms prepared using this process. The amorphous solid antibody dispersions display

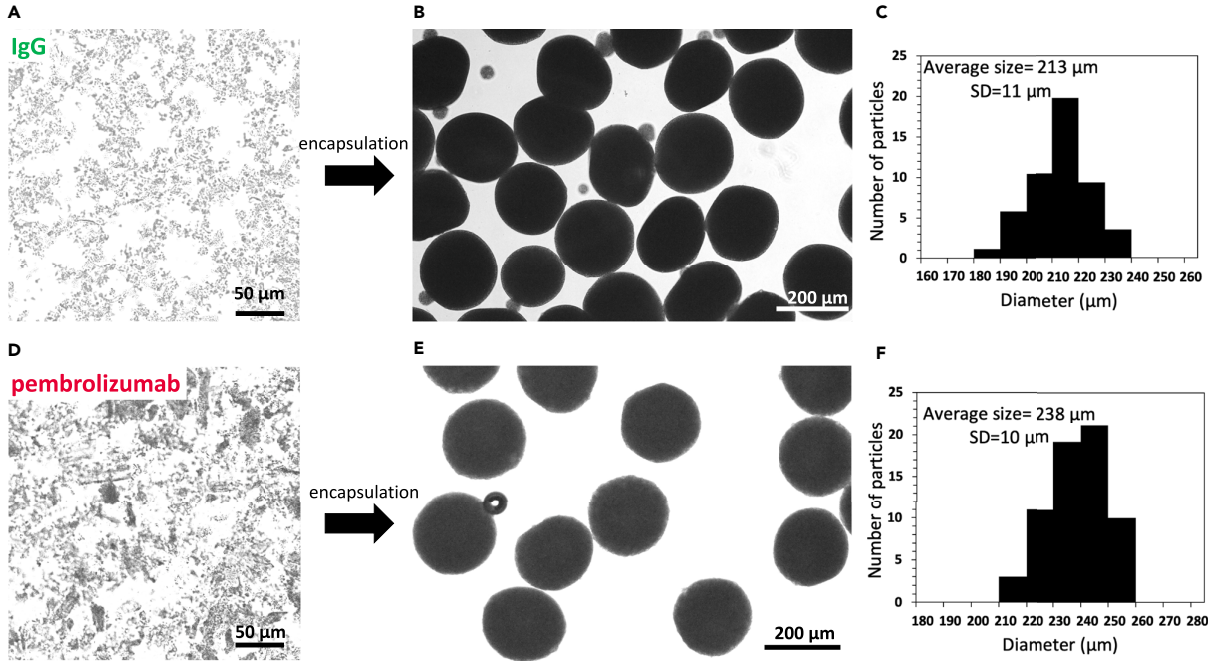

Figure 2. Encapsulation of amorphous solid antibody in alginate particles

(A–C) Microscopy images of amorphous solid IgG (A), IgG-laden alginate particles (B), and the corresponding particle size distribution (C). (D–F) Microscopy images of amorphous solid pembrolizumab (D), pembrolizumab-laden alginate particles (E), and the corresponding particle size distribution (F). Using amorphous solid antibody encapsulated in hydrogel particles can combine the stability benefits of a solid formulation with convenience of an injectable liquid formulation. Displayed IgG-laden particles have 250 mg/mL antibody particle loading and pembrolizumab-laden particles have 200 mg/mL antibody particle loading.

transient fractal structures. The antibody-laden alginate particles were spherical and opaque. These particles were stable in shape and morphology when kept in the storage buffer at 4°C for over 15 months.

There are several aspects that differentiate this dosage form and its corresponding encapsulating process from other microsphere dosage forms. The precipitation of the antibody to form amorphous solids can be adjusted for each desired antibody and can generally be developed with relative ease.[10,11,34] The narrow size distribution and uniform particles generated with microfluidic devices can benefit the translational research. Furthermore, the encapsulation process can be easily scaled up by use of multiple dispensers in each encapsulation device to produce antibody-laden particles with high throughput. Refer to the supplemental information for an estimation of the throughput of the device (refer to Figure S1). The throughput (given by the volumetric flow rate Q) of this encapsulation method is dictated by the flow rate of the pre-gel through the dispenser (refer to Figures S2–S4 and Table S1). The flow rate can be calculated based on pressure-driven flow for non-Newtonian power-law fluid in which shear stress (τ) and apparent viscosity (η) are described as

$$\tau = k\gamma^n \quad \text{(Equation 1)}$$

$$\eta = k\gamma^{n-1} \quad \text{(Equation 2)}$$

in which γ is the shear rate and k and n are the fluid constants. The volumetric flow is calculated as

$$Q = \frac{\pi R^3}{\frac{1}{n}+3}\left[\frac{(\rho aH)R}{2Lk}\right]^{\frac{1}{n}} \quad \text{(Equation 3)}$$

in which R, ρ, and a are the radius of the dispenser, suspension density, and centrifugal acceleration, respectively, while H and L are total height of the liquid and the dispenser length, respectively.

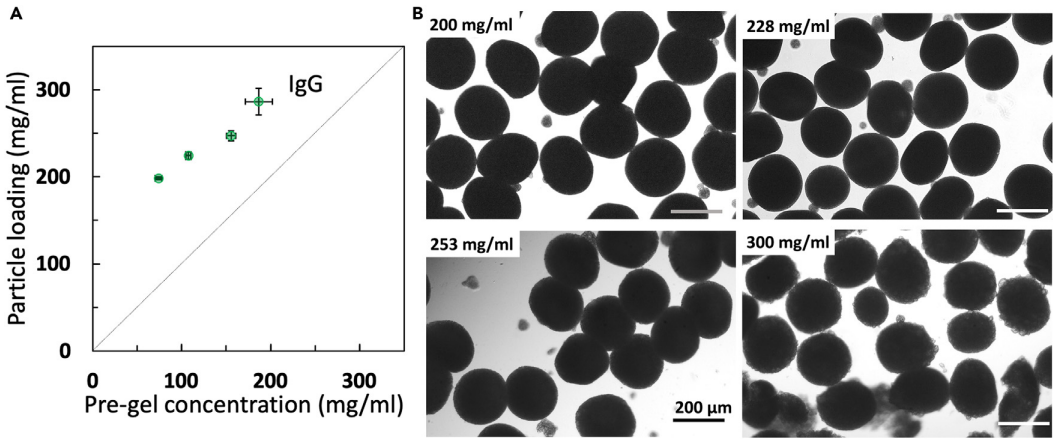

Figure 3. Amorphous IgG-laden alginate hydrogel particles at different concentrations

(A) Particle loading was defined as the antibody concentration within the particle after the production. Microscopy images of amorphous solid IgG-laden alginate particles at different particle loadings. Error bars represent mean ± standard deviation. Samples size n = 3. (B) Microscopy images of the IgG-laden particles at different concentrations. Scale bars are 200 µm.

The device throughput is limited by the high flow boundaries that allow a "dripping regime" (instead of fluid jet) and is illustrated in Figure S3.[33] The dimensionless numbers that govern this "dripping regime" are the Oh, We, and Bo numbers:[33]

$$Oh = \frac{\eta}{\sqrt{\rho R \sigma}} \quad \text{(Equation 4)}$$

$$We = \frac{\rho U^2 R}{\sigma} \quad \text{(Equation 5)}$$

$$Bo = \frac{\rho a R^2}{\sigma} \quad \text{(Equation 6)}$$

in which σ and U are surface tension and cross-sectionally averaged velocity of the pre-gel at the nozzle outlet, respectively.

Characterization of the amorphous solid antibody-laden hydrogel particles

Figure 3 displays the relationship between the IgG concentration in the pre-gel and the particle loading (antibody concentration within the particle after the production). The particle loading is important as it relates to the final formulation concentration (C_{form}) as

$$C_{form} = Particle\ loading * \varphi \quad \text{(Equation 7)}$$

where Φ is the effective particle volume fraction.

Particles with IgG concentration of up to 300 mg/mL were formed. The particle loading was measured by identifying the volume of the antibody-laden hydrogel particles (after removing the excess solution) and measuring the amount of the encapsulated mAb (using release experiment in simulated body fluid). Interestingly the particle loading was consistently higher than that of the pre-gel. This significant increase in the concentration can be attributed to the deswelling of the (IgG+alginate) mixture in the $CaCl_2$ gelation bath upon cross-linking.

As mentioned earlier, the amorphous solid antibodies dissolve outside the precipitation conditions (when injected). To find the appropriate formulate on conditions, the particles were immersed in varying concentrations of PEG to examine the onset of antibody dissolution. For this purpose, particles were equilibrated in corresponding PEG concentration for 24 h at 25°C before imaging. As illustrated in Figure 4, the amorphous solid IgG maintained its solid form at PEG concentrations above 5% w/w while pembrolizumab requires 7.5% w/v PEG, as observed by the change in the particle's transparency. Interestingly, the dissolution

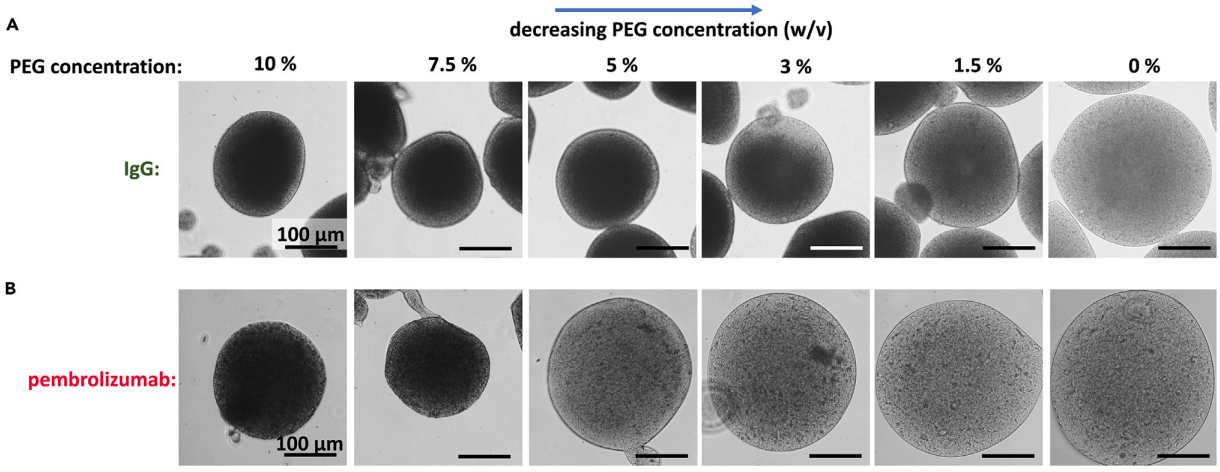

Figure 4. Amorphous solid antibody encapsulated within the alginate hydrogel particles dissolved outside of the precipitation conditions

(A and B) (A) IgG and (B) pembrolizumab-laden alginate particles were equilibrated at different PEG concentrations. Amorphous solid IgG started to dissolve at 3% w/v PEG which can be observed by change in the particle transparency and particle swelling. Amorphous solid pembrolizumab dissolved at 5% w/v PEG. Particles at 200 mg/mL antibody loading. Imaging conditions were kept constant. Scale bars are 100 µm.

of the polyclonal IgG is more gradual than the dissolution of the monoclonal pembrolizumab at varying PEG concentrations. We speculate that the gradual dissolution of IgG at different PEG concentrations is a result of heterogeneity in the polyclonal nature of amorphous solid IgG. Furthermore, we observed that when antibody molecules dissolve, the hydrogel particles become more hydrated as seen by the swelling of the particles. We speculate that swelling of the particles is caused by the increased osmotic pressure due to a high concentration of the dissolved antibody.

In vitro release and the released antibody quality attributes

As shown earlier, the amorphous solid antibody encapsulated within the particles was stabilized by the PEG in solution. Upon injection, the drop in the concentration of PEG triggers dissolution of the antibody. Figure 5 illustrates the release of the antibody from the hydrogel particles in the simulated body fluid[35] at 37°C. Simulated body fluid was prepared according to the established protocols, and contained among other salts, 7.996 g/L NaCl and 0.278 g/L $CaCl_2$ to simulate the effect of Na^+ and Ca^{2+} on the particle (ionically cross-linked alginate).[35] Both amorphous IgG and pembrolizumab dissolve within 2 min in the release experiment, which can be qualitatively observed by the change in transparency of the particles. For both IgG-laden and pembrolizumab-laden alginate particles, the encapsulated antibody was completely released from the hydrogel within the first 20 min (Figure 5C).

As the total amount of the encapsulated mAb was measured based on the amount of released antibody in simulated body fluid (SBF), which is an indirect method, special care was taken to ensure that antibody is not trapped. For this purpose, after the release experiment, the SBF solution was removed, and phosphate buffered saline was added which does not have calcium ions. In the absence of calcium, alginate particles were dissolved within days and the concentration of antibody in this solution was measured to confirm that the antibody was not trapped.

The relatively fast complete release assures that this formulation would not substantially affect the pharmacokinetics of the drug considering that the antibody absorption by the lymphatic system typically takes two or more days.[36,37] While we have only experimentally confirmed the fast and complete release for IgG and pembrolizumab, we speculate that similar release should be observed for other antibodies with similar characteristics (isoelectric point, self-interactions, and non-specific interactions). While our *in vitro* release profiles indicated that the dissolution followed by the diffusion can take place within few minutes, it is important to note that the release profile when using volumes typically administered in clinical settings (1–2 mL) injected in the SC space may result in different release profiles which need further investigation.

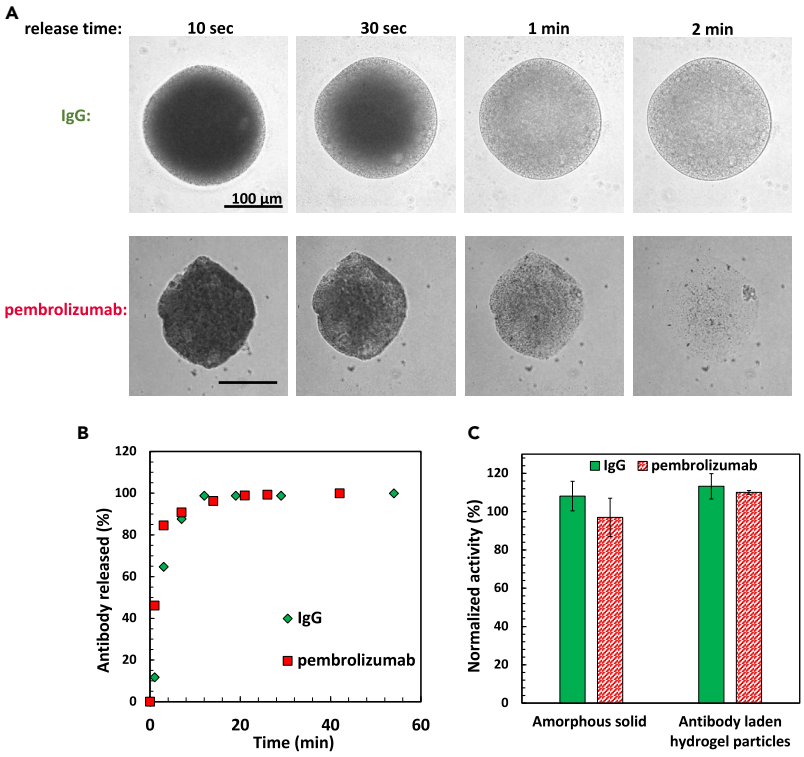

Figure 5. *In vitro* release of the encapsulated antibody from hydrogel particles in simulated body fluid

(A) Time-lapse microscopy imaging of the alginate hydrogel particles loaded with IgG *(top)* and pembrolizumab *(bottom)*. The samples were prepared at 250 mg/mL particle loading. Samples size n = 3; imaging conditions were kept constant.

(B) Antibody release profiles from particles at 37°C indicating that the encapsulated antibody is completely released from the particles within minutes.

(C) ELISA results for both IgG and pembrolizumab amorphous solid suspension and the amorphous solid antibody-laden hydrogel particles. Samples size n = 3. Error bars represent mean ± standard deviation. The ELISA results indicated that formation of amorphous solid antibody and later the encapsulation and release did not negatively affect the activity (potency) of the studied antibodies.

The activity (potency) of the IgG and pembrolizumab both as amorphous solid (before encapsulation) and after release from the particles was evaluated using an ELISA assay (Figure 5C). Results indicated complete activity indicating that the process of precipitation, concentration, encapsulation, and release was compatible with the antibodies.

For evaluating the stability of the amorphous solid IgG-laden hydrogel particles formulation, the particles at 200 mg/mL IgG loading were stored at 25°C for 100 days and compared with IgG solution prepared at the same concentration. Activity results (see Figure 6) indicated that while the activity of the IgG solution decreased, the amorphous encapsulated IgG maintained its complete activity. For the pembrolizumab, the stability of the formulation was evaluated at 4°C stored for 15 months. The size exclusion chromatography results for the studied mAb (Table 1) indicated no change in the monomer percent indicating stability of the formulation.

Injectability

Injectability of hydrogel particles through 27-gauge (ID = 210 µm) needle, commonly used in SC administration, is illustrated in Figure 7. Hydrogel particles were loaded in the syringe at different effective particle volume fractions (Φ), defined as

$$\varphi = \frac{\textit{Hydrogel particles' volume}}{\textit{Supsension volume}} = \frac{\textit{Supsension volume} - \textit{excess solution volume}}{\textit{Supsension volume}} \quad \text{(Equation 8)}$$

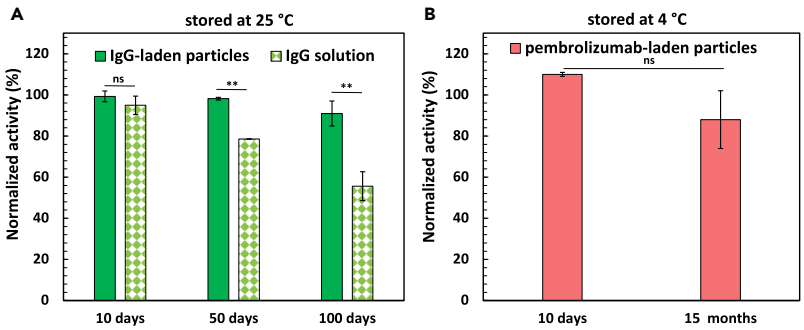

Figure 6. Stability of IgG and pembrolizumab evaluated using ELISA

(A) Improved stability of the amorphous solid IgG-laden hydrogel particles (compared to liquid formulation) observed by maintained activity. Both IgG-laden particles and IgG solution at 200 mg/mL and stored at 25°C.

(B) Amorphous pembrolizumab-laden particles at 200 mg/mL maintained their activity during 15 months storage at 4°C. Samples size n = 3. Error bars represent mean ± standard deviation. Data were compared using Student's *t* test; p values < 0.01 are indicated using ** and p values >0.05 are indicated as ns.

in which the "excess solution volume" was evaluated gravimetrically after being removed from the particle suspension. The density of the excess solution volume was measured earlier. The term "effective" was used to indicate that the reported volume fraction does not account for the changes to the specific volume of the particles caused by deswelling of the particles during the packing. It is for this reason that in suspensions of hydrogel particles the effective volume fraction can potentially reach $\Phi = 1$ or larger.[21]

Our results indicated that the soft nature of the hydrogels allowed high particle volume fractions to be packed inside the syringe and ejected from the needle. While hard spheres typically reach volume fraction up to $\Phi = 0.64$,[21] we illustrated ejection of $\Phi = 1.0$ volume fraction hydrogel particle through the needle. As can be seen in the figure, at volume fraction of $\Phi = 0.8$ (C_{form} = 200 mg/mL IgG) and $\Phi = 1.0$ (C_{form} = 250 mg/mL IgG), the injected hydrogel particles have a paste-like appearance. The injected particles at $\Phi = 0.6$ (C_{form} = 150 mg/mL IgG) and $\Phi = 0.8$ (C_{form} = 200 mg/mL IgG) were intact when visually compared to the particles before injection. For the $\Phi = 1.0$ sample, upon ejection from the needle, the turbidity of the suspension increased, which can suggest that the shear stress can cause erosion of the alginate particles upon injection at close to one volume fractions. Variables such as particle volume fractions and drug concentration within the particles can influence the shear viscosity profile and the injection force needed. However, determining the optimal conditions for achieving improved injectability in particulate-based formulations is beyond the scope of this work and should be considered for future research.

DISCUSSION

High-concentration formulation for biologics requires dosage forms adaptable to meet challenging stability requirements. In addition, the drug formulation processes must be gentle and fully compatible with labile nature of the therapeutic biologics and clinical research. Here we reported a novel method to formulate biologics that combines the stability of solid formulations with the convenience of liquid formulations which do not require reconstitution. The described process is based on precipitation of amorphous solid forms of the antibody and subsequent encapsulation within alginate hydrogel particles which have a track record of safe clinical use. This process can be easily applied to a variety of antibodies and was demonstrated for both mAb (pembrolizumab) and polyclonal antibody (immunoglobulin G) solutions with relative ease.

Table 1. Stability of amorphous pembrolizumab encapsulated in alginate hydrogel particles at 200 mg/mL evaluated using size exclusion chromatography

Sample	High molecular weight%	Monomer %	Low molecular weight %
Fresh	4.4	95.4	0.2
Stored for 15 months	4.6	95.2	0.2

Samples stored at 4°C. The low molecular weight % presents the fractionated monomers while the high molecular weight % indicates the antibody aggregates.

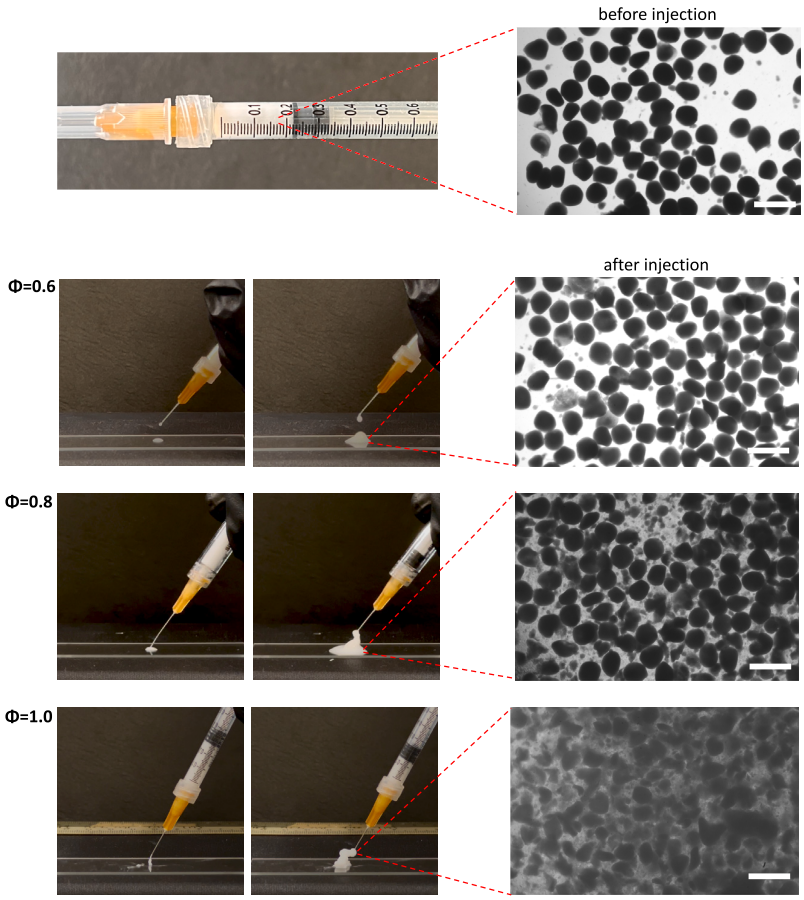

Figure 7. Injectability of amorphous solid IgG-laden alginate hydrogel particles through 27-gauge needle (ID = 210 μm) at varying particle volume fractions (Φ)

Alginate particles were at 250 mg/mL IgG antibody loading. Scale bars are 400 μm. Image acquisition conditions were kept constant.

Our dosage form has the benefits of encapsulating antibodies in highly stable amorphous solid eliminating the need for crystallization or lyophilization of biologics. This simple process is based on using a microfluidic device based on centrifugal extrusion that can be easily scaled up and produce particles in a continuous manner. Hydrogel particles of up to 300 mg/mL antibody loading were prepared for high-dose, low-volume formulations. These hydrogel particles' soft nature allowed high particle packings that enabled injectable high-concentration formulations. The *in vitro* release indicated complete antibody release from the alginate particles while the ELISA result confirmed the complete activity of the released antibodies. The stability studies indicated improved stability at room temperature for the IgG-laden hydrogel compared to the solution IgG. Furthermore, pembrolizumab formulated in alginate particles was stable during 15 months storage at 4°C. These new dosage forms and the results of this study can enable the full potential of biologics in treating cancer and auto-immune diseases by allowing the development of stable, concentrated, and versatile formulations with patient benefits that facilitate market adoption.

Limitations of the study

The current study lacks *in vivo* data for evaluation of bioavailability for SC administration of formulated biologics. Further characterization is needed for dissolution/release kinetics of bulk injections at volumes similar to dose used in humans. Additionally, the current study lacks control data for the activity of pembrolizumab aqueous formulation. Lastly, further characterization is needed to determine the optimal conditions for achieving improved injectability in particulate-based formulations.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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- EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS
- METHOD DETAILS
  - Materials
  - Antibody precipitation, encapsulation and characterization
  - Assessment of the released antibody quality
  - Viscosity of the pre-gel
- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2023.107452.

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AUTHOR CONTRIBUTIONS

All authors conceived the original idea and discussed the result. A.E. performed the experiments. A.E. analyzed the data with support from P.R. and P.S.D. A.E. wrote the manuscript with support from P.S.D. P.R., C.N.N, and P.S.D. revised the manuscript. C.N.N. and P.S.D. supervised the project.

DECLARATION OF INTERESTS

The authors have filed a patent application related to the research in this paper (application no. PCT/US2021/043916).

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
PembrolizumAb	Merck and Co.	N.A.
Human total IgG	Equitech-bio	VWR Catalogue # 103219-316
Chemicals, peptides, and recombinant proteins		
Sodium alginate	NOVAMATRIX	SKU: Product# 4200601
Critical commercial assays		
Human total IgG ELISA Kit	INVITROGEN	Catalog # BMS242

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to lead contact, Patrick Doyle (pdoyle@mit.edu).

Materials availability

There are restrictions to the availability of samples due to research agreement with Merck and Co.

Data and code availability

No standardized datatype data were generated in this study. This study did not generate new code. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

This work has not involved the use of human subjects or samples, nor has it use experimental models that require reporting of experimental model and subject details.

METHOD DETAILS

Materials

All chemicals used were of analytical grade and were used without any further purification. Low endotoxin level, ultra-pure sodium alginate was purchased from DuPont NovaMatrix. Low viscosity sodium alginate (apparent viscosity < 20 mPa.s^{-1}, molecular weight < 75kDa), and guluronic to mannuronic ratio of $\leq$ 1 was used for encapsulation. $CaCl_2$, and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) were purchased from Sigma. Poly(ethylene glycol) (PEG, 3350 KDa) was purchased from Hampton Research. Purified, humanized monoclonal antibody solution was provided by Merck & Co., Inc., Rahway, NJ, USA. Lyophilized IgG was purchased from VWR Scientific.

Antibody precipitation, encapsulation and characterization

For preparation of amorphous solid forms of anti-PD-1 antibody (pembrolizumab) *and human total IgG*, 666 μl of 50 mM HEPES containing 25 % w/v PEG was mixed with 333 μl of 40 mg/ml antibody in 20 mM histidine solution. Pembrolizumab was precipitated at pH 8.2 while IgG was precipitated at pH 7.2. Precipitation was carried out in batches at total volume of 1 mL, with each batch yielding ~13 mg of the antibody. The monoclonal antibody was kept frozen at 45 mg/ml in 20 mm L-His, pH 5.4 buffer and thawed right before the precipitation process. Solutions were prepared with distilled water and were filtered with a 0.22-micron filter. Precipitation mixture was kept at room temperature for 4 hrs while rotating at 12 rpm on a tube mixer. Amorphous solid antibodies were recovered from the batches by centrifugation at 1700 RCF for 30 minutes.

For preparation of the antibody alginate pre-gel suspension, at first sodium alginate powder was dissolved in the 15 % w/w PEG solution buffered with HEPES at 2 % w/v sodium alginate overnight and later filtered using 0.2-micron filter. Alginate was dissolved in the PEG solution to stabilize the antibody precipitates in the amorphous solid state. The sodium alginate solution was added to solid antibodies and mixed to generate a homogenous suspension and later the suspension was concentrated by centrifugation. Small quantities of the suspension were removed, and the antibody concentration was measured. Antibody concentration was measured using a Nanodrop UV-Vis spectrophotometer (the 280 nm absorbance method after 20-fold dilution in PBS). Centrifugation was continued until the suspension reached the desired antibody concentration. This final suspension was used as the pre-gel to form the antibody-laden alginate hydrogel particles.

For preparation of antibody-laden alginate particles, the pre-gel suspension was filled inside a simple microfluidic device made from a plastic syringe (with the plunger removed) connected to a 30-gauge (ID=159 μm, OD=312 μm) blunt-tip dispenser.[33] Solution consisting of 40 mM $CaCl_2$, 15 % w/v PEG 3350 (for the stabilization of solid antibodies), and 50 mM HEPES pH 7.2 was used for crosslinking. The crosslinking solution was filled inside a 15 ml centrifuge tube to form the $CaCl_2$ bath. The distance from the tip of the dispenser to the $CaCl_2$ bath was ~2 mm. The device was centrifuged for 10 – 30 minutes at 400 RCF. The pre-gel flowed through the dispenser due to the centrifugal acceleration to form the droplets and subsequently forming hydrogel particles after impact. Upon completion the bath solution was replaced with 15 % w/v PEG, 50 mM pH 7.2 HEPES (storage buffer) and particles were resuspended.

For particle antibody loading measurement, (antibody+alginate) pre-gel suspensions were prepared, and the antibody concentration was measured using the protein 280 nm absorbance method. Later particles were formed using the same suspensions. Hydrogel particles were redispersed, and the excess aqueous solution was wiped using a tissue paper and the mass of the antibody-laden hydrogel was measured. The total amount of the encapsulated mAb was measured based on the amount of released antibody in phosphate buffer saline to calculate the particle loading.

Simulated body fluid was prepared to mimic the amount of monovalent and divalent ions in the SC environment, based on the literature[35] at sodium chloride 7.996 g/L, sodium bicarbonate 0.350 g/L, potassium chloride 0.224 g/L, potassium phosphate dibasic trihydrate 0.228 g/L, magnesium chloride hexahydrate 0.305 g/L, 40 ml of 1 M hydrochloric acid, $CaCl_2$ 0.278 g/L, sodium sulfate 0.071 g/L , and tris(hydroxymethyl) aminomethane 6.057 g/L.

To evaluate the release of mAb from the hydrogel, ~ 50 μl of the antibody-laden alginate hydrogel particles were moved to a 2 ml tube. Later at time zero the excess buffer was replaced by the pre-warmed (37°C) simulated body fluid, and small volumes of the sample (10μl) were removed at different time intervals to measure the antibody concentration using the 280 nm absorbance method.

For filling of the syringes with antibody-laden alginate particles and to reach high effective particle volume fractions the syringes were backfilled. Later the syringes were capped and centrifuged at 3000 rcf for 10 minutes and the excess solution was removed (from the tip) using a micropipette.

Assessment of the released antibody quality

Bioanalytical studies were carried out to evaluate the quality of the released anti-PD-1 antibody. Size Exclusion Chromatography (SEC) was used to determine the amount of the antibody monomer and aggregates for the pembrolizumab-laden particles. Multi-angle light scattering (MALS) was utilized to determine the molecular weights. For this purpose, Waters Acquity UPLC H-Class Bio system (attached to a Wyatt UP LS laser and UP tRex detector) with a Waters Acquity BEH200 SEC column to separate molecules based on their hydrodynamic radius was utilized. Resolved peaks were detected by the absorbance at 280 nm, light scattering intensity, and the differential refractive index. SEC experiments were carried out under isocratic conditions at a flow rate of 0.5 mL/min in a buffer composed of 50 mM phosphate, 450 mM arginine at pH 7.0.

ELISA bioassays were carried out to identify the anti-PD-1 antibody and human total IgG bioactivity using binding assays. To evaluate the ability of the antibody to bind to the other ligand with correct receptors, the reference material and test samples were serially diluted and mixed with an equal volume of the ligands

before transfer to ELISA plates. The levels of bound ligand to the ELISA plate were detected by conjugation with streptavidin and chemiluminescence substrate. Luminescence was measured using a microplate reader and resulting inhibition response curves were analyzed with curve fitting software (e.g., SoftMax Pro). Biological potency is expressed as % relative potency of the reference sample. The ELISA assay for the IgG was carried out based on the manufactures (Invitrogen) protocol. Samples were measured in triplicate.

Viscosity of the pre-gel

The viscosity of the pre-gel was measured for the IgG pre-gel sample at 200 mg/ml. The viscosity was measured using a microfluidic slit rheometer referred to as VROC (Viscometer-Rheometer-on-Chip, Rheosense Inc) at high shear rates. The specific microfluidic device used in this study, mVROC Type E10 chipset, consists of a rectangular cross-section channel (width = 100 μm and depth = 200 μm) containing four mounted pressure sensors.

QUANTIFICATION AND STATISTICAL ANALYSIS

The data of the experiments were presented as mean ± standard deviation (SD). Data were compared using students t-test and P values <0.01 are indicated using ** and P values > 0.05 are indicated as ns. Statistical details of experiments can be found in the figure legends.