Submission ID #: 62234
Scriptwriter Name: Bridget Colvin
Project Page Link: https://www.jove.com/account/file-uploader?src=18981418

Title: Injectable Supramolecular Polymer-Nanoparticle Hydrogels for Cell and Drug Delivery Applications

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Author Questionnaire

1. Microscopy: Does your protocol demonstrate the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or similar? **N**

2. Software: Does the part of your protocol being filmed demonstrate software usage? **N**

3. Interview statements: Considering the Covid-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

☒ Interviewees wear masks until the videographer steps away (≥6 ft/2 m) and begins filming. The interviewee then removes the mask for line delivery only. When the shot is acquired, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

3. Filming location: Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Protocol Length
Number of Shots: **35**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Catherine M. Meis**: Our protocol facilitates the formulation of polymer nanoparticle hydrogels for their use as biomaterials. We hope researchers will develop this material for translational applications and to explore basic biological questions **[1]**.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

REQUIRED:

1.2. **Abigail K. Grosskopf**: PNP hydrogels are easily injected through small needles and catheters and quickly self-heal after injection, allowing the non-invasive, controlled delivery of drugs and cells over long timescales **[1]**

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

OPTIONAL:

1.3. **Santiago Correa**: This technology pushes the boundaries for localized therapy and extended drug release, with implications for wide-ranging conditions from cancer to tissue regeneration to passive immunization **[1]**.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Protocol

2. Nanoparticle (NP) Synthesis

2.1. To synthesize nanoparticles by nanoprecipitation, add 50 milligrams of PEG-PLA (peg-P-L-A) polymer to an 8-milliliter glass scintillation vial **[1]** and add 1 milliliter of acetonitrile to the vial **[2]**. Vortex to fully dissolve **[3]**.

2.1.1. WIDE: Talent adding polymer to vial

2.1.2. Talent adding acetonitrile to vial

2.1.3. Vial being vortexed

2.2. Next, add 10 milliliters of ultrapure water to a 20-milliliter glass scintillation vial with a small stir bar **[1]** and place the vial on a stir plate set to 600 revolutions per minute **[2]**.

2.2.1. Talent adding water to vial with stir bar

2.2.2. Vial on plate being stirred

2.3. Use a 200-microliter pipette to add 1 milliliter of the polymer solvent solution dropwise to the vial of water **[1]**. Core-shell nanoparticles will form as the polymer solvent solution is rapidly dispersed throughout the water **[2]**.

2.3.1. Solution being added to vial *Videographer: Important step*
2.3.2. Shot of vial during nanoprecipitation *Videographer: Important step*

2.4. Verify the particle size by dynamic light scattering according to standard protocols **[1]**.

2.4.1. Talent adding sample to instrument

2.5. Then transfer the nanoparticle solution into a centrifugal filter unit to concentrate the solution to less than 250 microliters **[1-TXT]** and resuspend the nanoparticles in an appropriate buffer **[2]**.

2.5.1. Talent adding solution to filter unit **TEXT: 1 h, 4500 x *g*, RT**
2.5.2. Shot of NP if visible, then buffer being added to unit, with buffer container

visible in frame

3. Hydrogel Formulation and Rheological Property Measurement

3.1. To prepare the hydrogel, add 333 milligrams of 6% of HPMC-C-12 (H-P-M-C-C-twelve) stock solution to a 1-milliliter Luer lock syringe **[1-TXT]** and add 500 microliters of the 20% nanoparticle stock solution and 167 microliters of PBS to an 8-millililter vial **[2]**.

3.1.1. WIDE: Talent adding solution to syringe *Videographer: Important step* **TEXT: HPMC-C_{12}: dodecyl-modified hydroxypropylmethyl cellulose polymer**

3.1.2. Talent adding NP and PBS to tube, with NP and PBS containers visible in frame

3.2. After mixing, use a needle to fill another 1-milliliter Luer lock syringe with the diluted nanoparticle solution **[1]** and attach the two syringes to an elbow mixer **[2]**.

3.2.1. Talent loading syringe *Videographer: Important step*

3.2.2. Syringes being attached *Videographer: Important step*

3.3. Mix the two solutions for approximately 60 cycles **[1]** until a homogeneous, opaque white hydrogel material has formed **[2]**.

3.3.1. Solutions being mixed *Videographer: Important/difficult step*

3.3.2. Shot of opaque white hydrogel *Videographer: Important step*

3.4. To measure the rheological properties of the formulated hydrogel, inject the appropriate volume of hydrogel according to the selected geometry gap into the center of a serrated rheometer plate **[1-TXT]** and use oscillatory and flow tests to measure the mechanical properties of the sample **[2]**.

3.4.1. Talent injecting hydrogel onto plate *Videographer: Important/difficult step* **TEXT: e.g., 600-700 microliters material for 20-mm plate with 700-micron gap**

3.4.2. Representative shot of Talent performing one test

4. In Vitro Drug Release Characterization

4.1. To characterize drug release from the hydrogel, first prepare glass capillaries by using epoxy to seal one end of each tube **[1]**.

4.1.1. WIDE: Talent adding epoxy to tube end

4.2. When the epoxy has set, use a 4-inch, 22-gauge hypodermic needle to inject 100-200 microliters of hydrogel into a minimum of three tubes per sample **[1]** and carefully add 200-300 microliters of PBS onto each volume of hydrogel **[2]**.

4.2.1. Hydrogel being added to tube
4.2.2. PBS being added to hydrogel

4.3. At the appropriate time points, according to the anticipated time scale of drug release, use a needle to carefully remove the PBS from each capillary without disturbing the hydrogel surface **[1]** and add a fresh volume of PBS **[2]**.

4.3.1. PBS being removed *Videographer: Important step*
4.3.2. PBS being added *Videographer: Important step*

4.4. At the completion of the study, analyze the collected PBS aliquots with an appropriate method to quantify the amount of drug released at each time point **[1]**.

4.4.1. Talent adding sample to 96-well plate with ELISA kit visible in frame or similar representative shot

5. Thermal Stability of Gel-Encapsulated Insulin Characterization

5.1. To characterize the thermal stability of gel-encapsulated insulin, load both insulin and thioflavin T into the hydrogel as demonstrated **[1]** and use a 21-gauge needle to inject 200 microliters of the cargo- and probe-loaded hydrogel into at least three wells of a black, 96-well plate per sample **[2]**.

5.1.1. WIDE: Talent mixing hydrogel, with hydrogel, insulin, and ThT containers visible in frame
5.1.2. Talent injecting hydrogel into well(s)

5.2. Then seal the plate with an optically clear adhesive plate seal to prevent evaporation **[1]** and insert the plate into a plate reader equipped with temperature control, shaking, and a kinetic read programming **[2]**.

5.2.1. Plate being sealed
5.2.2. Talent loading plate onto plate reader

6. Cell Viability Assessment

6.1. To assess hydrogel-encapsulated cell viability, use a 21-gauge needle to inject 150 microliters of hydrogel containing the appropriate concentration of cells into each of three wells per sample in a clear bottom, 96-well plate **[1]** and add 100 microliters of the appropriate cell medium onto each volume of hydrogel **[2]**.

6.1.1. WIDE: Talent adding hydrogel to well(s)

6.1.2. Talent adding medium to well(s), with medium container visible in frame

6.2. On Day 1 of culture, replace the supernatant on each hydrogel at the appropriate time point for each sample group with 50 microliters of 2-millimolar calcein AM (A-M) solution **[1]**.

6.2.1. Calcein AM being added to hydrogel(s), with calcein container visible in frame

6.3. After a 30-minute incubation, image the center of each well by confocal microscopy **[1]**.

6.3.1. LAB MEDIA: Figure 5 RGD Day 1 image

7. Cell Settling Assessment

7.1. To evaluate the ability of the encapsulated cells to settle in a syringe before injection, dilute the cells of interest to 1 x 10^6 cells/milliliter in PBS concentration **[1]** and stain the cells with 50 microliters of 2-millimolar calcein AM for 10 minutes at room temperature **[2]**.

7.1.1. WIDE: Talent adding PBS to cells, with PBS and cell containers visible in frame
7.1.2. Talent adding calcein AM to cells, with calcein AM container visible in frame

7.2. At the end of the incubation, mix the cells with 500-700 microliters of hydrogel as demonstrated **[1]** and use a 21-gauge needle to inject 100-200 microliters of cell-containing hydrogel into the bottom of at least one cuvette per sample **[2]**.

7.2.1. Talent mixing cells, with hydrogel and cell containers visible in frame
7.2.2. Talent adding hydrogel to cuvette

7.3. Then image the cuvettes lying flat on their sides on the stage of a confocal microscope immediately after injection **[1]** and at 1 and 4 hours after seeding to observe whether the cells have settled in the hydrogel **[2]** or whether they have remained suspended **[3]**.

7.3.1. LAB MEDIA: FIGURE 5C *Video Editor: please 15 min images*
7.3.2. LAB MEDIA: Figure 5C *Video Editor: please sequentially emphasize 1- and 4-hour 1:1 images*
7.3.3. LAB MEDIA: Figure 5C *Video Editor: please sequentially emphasize 1- and 4-hour 1:5 images*

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see? Please list 4 to 6 individual steps.
2.3., 3.1.-3.4., 4.3.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success? Please list 1 or 2 individual steps from the script above.
3.3., 3.4. Preparing all hydrogel components to the specified quality and carefully mixing gel components are both necessary to produce PNP hydrogels with consistent and reproducible mechanical properties as measured by rheology.

Results

8. Results: Representative Polymer Nanoparticle (PNP) Hydrogel Release Kinetics, Cargo Stability, and Cell Viability

8.1. The shear-thinning **[1]** and self-healing capabilities of the gel **[2]** can be observed using flow sweep and step-shear protocols, respectively **[3]**.

8.1.1. LAB MEDIA: Figures 2A and 2B *Video Editor: please emphasize Figure 2A*
8.1.2. LAB MEDIA: Figures 2A and 2B *Video Editor: please emphasize Figure 2B*
8.1.3. LAB MEDIA: Figures 2A and 2B

8.2. Characterization of the storage and loss moduli using an oscillatory shear frequency sweep experiment in a linear viscoelastic regime at frequency ranges from 0.1-100 rad/second reveals the solid-like properties **[1]**.

8.2.1. LAB MEDIA: Figure 2C

8.3. There should typically not be a crossover of the shear storage and loss moduli observed at low frequencies for stiffer formulations **[1]**, while crossover events can be expected for weaker hydrogel formulations **[2]**.

8.3.1. LAB MEDIA: Figure 2C *Video Editor: please emphasize blue data lines*
8.3.2. LAB MEDIA: Figure 2C *Video Editor: please emphasize pink data lines*

8.4. Varying the polymer content of the PNP (P-N-P) hydrogels **[1]** can have a direct impact on the diffusion of cargo through the polymer network and the rate of release from the materials **[2]**.

8.4.1. LAB MEDIA: Figure 3 *Video Editor: please emphasize syringes in Figure 3A*
8.4.2. LAB MEDIA: Figure 3 *Video Editor: please sequentially emphasize green, pink, and blue data lines in Figure 3B*

8.5. PNP hydrogels can also stabilize cargo that is susceptible to thermal instability **[1]**, considerably extending the cargo shelf life and reducing the reliance on cold chain storage and distribution **[2]**.

8.5.1. LAB MEDIA: Figure 4
8.5.2. LAB MEDIA: Figure 4 *Video Editor: please emphasize blue and green data lines*

8.6. The inclusion of integrin motifs can be useful for adapting PNP hydrogels for cellular therapies **[1]**. Encapsulated cells can be fluorescently labeled to facilitate their visualization **[2]** and quantification **[3]**.

8.6.1. LAB MEDIA: Figures 5A and 5B
8.6.2. LAB MEDIA: Figures 5A and 5B *Video Editor: please emphasize Figure 5A*
8.6.3. LAB MEDIA: Figures 5A and 5B *Video Editor: please emphasize Figure 5B*

8.7. For example, formulations lacking adhesion sites will have a low cell viability, as encapsulated cells fail to proliferate **[1]** compared to cells encapsulated in formulations with adhesion motifs **[2]**.

8.7.1. LAB MEDIA: Figures 5A and 5B *Video Editor: please emphasize No RGD images and data bar*
8.7.2. LAB MEDIA: Figures 5A and 5B *Video Editor: please emphasize RGD images and data bar*

Conclusion

9. Conclusion Interview Statements

9.1. **Catherine M. Meis**: We are still exploring how changes in the formulation affect the rheological characteristics and dynamic mesh of the polymer matrix. We also use FRAP to study the diffusion of molecules within the hydrogel **[1]**.

9.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

9.2. **Abigail K. Grosskopf**: These materials can be used to ask new biological questions about how sustained delivery from these materials might affect drug delivery, vaccine development, or cancer immunotherapy **[1]**.

9.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera