

Editorial comments:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

We have reviewed the manuscript and believe it is free of editing errors.

2. Please provide the complete address of all the affiliations.

We have updated the manuscript with the complete addresses of affiliations of each author.

3. Please revise the text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).

We have removed all personal pronouns.

4. Please ensure that when giving a reference in the text, the corresponding number from the reference list must appear superscripted without a space after the word/group of words it applies to but before any punctuation (in the case of et al., place the superscripted number after et al. but before other punctuation).

The punctuation has been corrected throughout.

5. Line 36-38: Please revise the following lines to avoid previously published work: Paul, Arghya, Anwarul Hasan, Hamood Al Kindi et al. "Injectable Graphene Oxide/Hydrogel-Based Angiogenic Gene Delivery System for Vasculogenesis and Cardiac Repair", ACS Nano; Kim, Kyung Hoon; Zheng, Nan; Gabrielson, Nathan P.; Cheng, Jianjun Advanced Materials (Weinheim, Germany) (2013), 25 (22), 3063-3070CODEN: ADVMEW; ISSN:0935-9648.

We believe these lines avoid these previously published works. In these lines, we are introducing the general scope of our manuscript.

6. Lines 78- 80: Please revise the following lines to avoid previously published work: Bioeng Transl Med. 2019 Oct 22;5(1):e10147. doi: 10.1002/btm2.10147. eCollection 2020 Jan.Injectable supramolecular polymer-nanoparticle hydrogels enhance human mesenchymal stem cell delivery. Abigail K Grosskopf 1, Gillie A Roth 2, Anton A A Smith 3, Emily C Gale 4, Hector Lopez Hernandez 3, Eric A Appel 3

The text has been edited to avoid previously published work. The noted work is also cited in the text.

7. Please use standard abbreviations for SI Units when the unit is preceded by a numeral. Abbreviate liters to L to avoid confusion. Examples: 10 mL, 8 µL

The abbreviations have been changed to the correct format.

8. Line 135: Please add more details to your protocol steps. Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action.

The following lines have been added or updated to explain how the DLS step is performed:

“Note: This procedure is specifically for using a Wyatt DynaPro Plate Reader DLS with the Wyatt DYNAMICS software package.”

“Measure the hydrodynamic radius and polydispersity of each sample with a DLS plate reader using preset protocol options in the DYNAMICS software package. As an example of a typical protocol, set the data collection parameters to acquire 5-10 DLS measurements of 2-5 s duration per acquisition and then report a mean particle size and distribution per well, calculated using the globular proteins model.”

9. Please convert centrifuge speeds to centrifugal force (x g) instead of revolutions per minute and italicize g in centrifugation speeds. Example: 1,000 x g

The centrifuge speeds have been converted and denoted as requested.

10. Line 253: Please elaborate on how the mentioned tests are performed.

Additional details were added to further describe the methods of the mechanical tests for testing viscoelasticity and flow properties. Additionally, a reference with explanations of these tests was added.

11. Line 275: Please specify how the sealing step is performed.

The following text has been added to clarify this step:

“Seal one end of each tube by using a disposable spatula or pipette tip to push a small amount of epoxy into the end of the tube to form a plug. Allow epoxy to set per manufacturer’s recommended time.”

12. Line 281: Please mention the specifications of the hypodermic needle.

The needle specifications have been added to both the text and the table of materials.

13. Line 310-312: Please provide the details for the analysis techniques mentioned. Appropriate citations would suffice.

The appropriate detection method will vary depending on the drug of interest. We have added relevant citations with protocols that could be adapted for this particular step.

14. Line 353: Please mention how the arbitrary signal threshold is defined. Insert references if required.

We have added references as well as the following text to address the definition of a signal threshold:

“Note: For the representative data shown below, threshold was defined as 750,000 arbitrary fluorescence units (AFU). This value was chosen to be above the measured baseline while still sufficiently capturing the onset of aggregation indicated by a sharp fluorescent signal increase.”

15. Line 400: Please mention the centrifugal conditions (speed, time)

The centrifugal conditions will vary depending the chosen cell type for the experiment. We have added the following text for clarification of the procedure:

“Note: The recommended speed and duration to centrifuge each specific cell type is typically provided in the product documentation.”

16. Line 412: Please elaborate on image stitching. Cite references wherever necessary.

Additional details were added to explain the image processing. Additionally, citations were added to explain the algorithm that performs image stitching and maximum intensity projections in FIJI image processing software.

17. Please Do not use the &-sign or the word “and” when listing authors. Authors should be listed as last name author 1, initials author 1, last name author 2, initials author 2, etc. End the list of authors with a period. Example: Bedford, C. D., Harris, R. N., Howd, R. A., Goff, D. A., Koolpe, G. A. Quaternary salts of 2-[(hydroxyimino)methyl]imidazole. Journal of Medicinal Chemistry. 32 (2), 493-503 (1998).

We have corrected the reference formatting using the Jove style in our reference manager and also manual updates.

18. Please do not use any abbreviations for the journal titles and book titles.

We have corrected the reference formatting using the Jove style in our reference manager and also manual updates.

19. Figure 2: Please use the multiplication dot for compound units. Examples: N·m, m/s, eV·s/rad, m²·kg·s⁻³A⁻², m²·kg/s³

The multiplication dot has been used in the updated figure.

20. Figure 5: Please include a scale bar for all images taken with a microscope to provide context to the

magnification used. Please define the term RGD in the figure or the text (5a). Please make sure that the X-axis is defined correct (5d).

The requested edits have been made in the updated figure. RGD has been defined in the text.

21. Please revise the table of materials in alphabetical order.

The table has been alphabetized.

Reviewer #1:

Well written and full of details

Thank you for the positive comment. We hope this method will be descriptive and useful to those seeking to formulate these types of hydrogels.

Reviewer #2:

This manuscript describes the formulation and characterization of PNP hydrogels for use as biomaterials. These materials have potential utility for drug delivery, stabilization of biomolecules, and cell encapsulation and delivery. The authors do a great job of thoroughly describing representative protocols associated with these applications.

Thank you for the kind remarks. We hope and anticipate that these methods will help other researchers formulate and test the materials described as well as other soft biomaterials.

Major Concerns:

I have no major concerns.

Minor Concerns:

There are a few small typos throughout the manuscript. In addition, some lines are highlighted in yellow, and it is not clear why.

The highlighted lines are to indicate which sections should be adapted for video scripting. We believe we have corrected any typos after careful editing; thank you for bringing this to our attention.