nature portfolio

Peer Review File

Porous Hierarchically Ordered Hydrogels Demonstrating Structurally Dependent Mechanical Properties

Corresponding Author: Professor Robert Hickey

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

This manuscript has been previously reviewed at another journal. This document only contains information relating to versions considered at *Nature Communications*.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The work by Elisabeth et al. reported on the porous hydrogel assembly from ABA block polymers, exhibiting a hierarchical structure with an extremely low elastic modulus, strain-hardening behavior and high elasticity with a low hysteresis. The experiments are carefully conducted, and a large amount characterization are performed. The work is nice, while some data require further analysis. The referee recommends publishing this work after a major revision. The detailed comments are as follows:
(1)Page 4, line 77-84. Sufficient citations should be inserted to support the demonstration of differences between the current work and previous works.
(2)Page 14, line 317-320. Although the authors mentioned that the hydrogels exhibit strain-hardening behavior, no detailed analysis of this behavior was carried out in this manuscript.
(3)Page 14, line 309. The authors stated that the reported porous hydrogels are brittle; however, a referenced work (Chem. Mater. 2022, 34, 10995–11006) demonstrates that the triblock porous hydrogels are highly resilient.
(4)The corresponding diagrams in SI are necessary to be mentioned in the main text. e.g. Figure S5, S6.
(5)Page 3, line 60-66. The references are necessary to mention at the annotation of Figure 1a because the morphological images (i, ii, iii) were clearly captured from other studies.
(6)Figure 5b There are missing necessary annotation to distinguish what the red and blue symbols represent, respectively.

Reviewer #3

(Remarks to the Author)
The revised manuscript is suitable to be published.

Reviewer #4

[Editorial Note: Reviewer #4 was recruited to look over the responses to reviewer 2 from a previous round of review]

(Remarks to the Author)
The authors Lloyd et al. proposed a bottom-up method to produce highly porous hydrogel fibers that resemble extracellular matrices both structurally and mechanically. The underling mechanism for the formation of the microstructure of the hydrogel is said to be the liquid-state spinodal decomposition. My recommendation is that the current work should not be published in Nature Communications.

The critical concerns of the reviewer 2 except comment 4 have not been addressed.
The authors claimed that the comment 3 is beyond the scope of the current work and will do it in future. For the comment 1, the phase diagram is not improved.

From my experience, the current phase diagram is quite artificial. My specific comments are as follows:

1. The authors hypothesize that spinodal decomposition is the underlying mechanism for the microstructure formation. However, this can only be applied for the LIQUID state.
The microstructure observed in experiment is a SOLID structure, at least a soft GEL state rather than a LIQUID structure. In

the real case, one has to consider the gelation/solidification in the phase diagram.
A sketch for the transformation of a binary polymer liquid solution into the gel state has been shown in Fig. 12, Hajime Tanaka 2000 J. Phys.: Condens. Matter 12 R207.
The current work focuses on "hydrogel"; it is very empirical when applying a liquid state phase diagram for the formation of hydrogel.

2. Even for the liquid-state phase diagram, there is a big problem. I guess the blue line in Fig.4 is the spinodal line; the authors should clearly indicate the meaning of different lines in Fig.4.
By using the degree of polymerization Np=4436, it is impossible to produce the binodal line according to the Flory-Huggins theory, while the spinodal line can be estimated analytically.

3. When producing the liquid phase diagram, the Flory parameter χ should be paid attention carefully. For me, the current parameters χ are arbitrarily selected without too much physical meanings.

I have also looked at the comments of reviewer 1 and the response letter of the authors.

I agree with reviewer 1 that designing highly extendable, reversibly deformable, and extremely soft hierarchical hydrogels has already been published in the previous work by Prof. Hickey, see Nature Communications 10, 3855, 2019. I have compared the current manuscript with the previous one; the characterization method is quite similar. Thus, the novelty of the current work is limited.

In all, I feel that the mechanism for the micro-structure formation of hydrogel presented by the authors is quite artificial. The present manuscript with some revision may be suited for other journals, like Macromolecules.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
I have read the revised manuscript, and the authors have already addressed the referees' comments, as well as answered Reviewer 4's comments. I recommend that this work be published in this journal.

Reviewer #4

(Remarks to the Author)
I am not convinced that the very simplified mechanism (Figure 4) proposed by the author
is sufficient to address the formation of hydrogels. From my opinion, this is a hand-waving theory which cannot be verified in physical modelling and is far from real physics of hydrogels.

With such a high degree of polymerization, 4436, in the Flory-Huggins theory, as presented by the authors, it is almost impossible to capture the spinodal decomposition in a physical modeling, for example, the Cahn-Hilliard model. The problem is that due to the large degree of polymerization, the polymer-rich phase and the polymer-lean phase reach the binodal line asynchronously; the binodal line is not shown by the authors in Figure 4. Suffice to say, when the polymer-lean phase reaches the binodal line,
the polymer-rich phase still needs a much longer time to arrive at the binodal
line due to the distinct entropy contributions of polymer and solvent arising from the high degree of polymerization. If only considering the liquid-state phase separation, as proposed by the authors, the mass is not conserved any more for such a high degree of polymerization.
Usually, a very small value for the degree of polymerization (for example, 5, 10, 20, some values like this) is adopted in the simulation to capture the spinodal decomposition (see J. Chem. Phys. 158, 214903 (2023) and similar works).
One possible way to amend this is to add the gelation mechanism in the theory, as suggested in my previous report. The authors have to address this mechanism before publication.
The current mechanism in Figure 4 is far from real physics and is too simple with only considering the liquid phase diagram. This simplified theory is at the time stage of 1940s and is not the-state-of-the-art.

The authors should also improve the writing style and present the figures in a more clear way.
For example, in the caption of Figure 4,
"a)...., the lines show the predicted..", which lines? there are several lines, light blue, dark blue, cyan? Such a presentation style is very ambiguous.

Version 2:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The authors have fully responded to the reviewers' comments. I recommend the publication of the paper.

Reviewer #4

(Remarks to the Author)
I am grateful to the authors for their efforts in writing the rebuttal letter.
However, there is no improvement of the phase diagram in Figure 4 at all.
I mean for the large degree of polymerization used by the authors, the BINODAL line is impossible, not the spinodal line argued by the authors. The current phase diagram is nothing related to hydrogel. It is a kind of artificial phase diagram without physical meanings. It has only arbitrary spinodal lines which cannot be verified in the experiments. The binodal line is missing, when the authors emphasize on the importance of the equilibrium rather than the kinetics. Experiments should be performed to confirm the binodal as well as the spinodal lines.
In all, the current manuscript has serious flaws in the theory and is not suited for Nature Communications.

Reviewer #5

[Editorial Note: Reviewer #5 was recruited to additionally assess the responses to concerns raised by other reviewers]

(Remarks to the Author)
In my review of the revised manuscript I also have concerns about the applicability of the simplified model (as Rev. 4). The mechanism of macrophase separation between PS-PEO-PS and water is not well understood.
In addition to this, I find the explanation of the scattering data not consistent with the applied model (interactions between spheres described by a Percus-Yevick hard sphere interaction potential). Specifically, in the SI the authors provide some fits to the model (Figure S4) and discuss the parameters (domain spacing, micelle diameter and volume fraction) in Figure S5. However, the PY model provides the domain spacing (from the maximum of the structure factor) which is ~ 80 nm as discussed, the sphere radius (from the first minimum of the form factor, at qR=4.493) and the hard sphere radius. In addition, it provides the volume fraction of spheres and requires some polydisprersity. The problem is that the reported values do not match with the experiment. For example, the first minimum in the form factor is at ~ 0.1 nm-1 giving a sphere radius of ~45 nm, i.e. much larger than the reported values. In addition, the hard sphere radius is not clearly reported. Lastly, polydispersity is not applied, although clearly needed (minima are not clearly seen).
One related point: the model does not fit the whole scattering curve – but a part of it (Figure S4). What is the reason?
Another point relates to the stress-strain data. The stress-strain data of Figure 5 show strain hardening. It is mentioned that the difference in the onset of strain hardening is due to the pore size and pore microstructure…highlighting new avenues for controlling mechanical responses..". It is not clear how the two are related.
A lesser point: ref [35] is in error.
For the above reasons and the comments of Rev.4 I do not feel that the work can be published by Nature Communications. I could suggest, instead, a more specialized journal (after the points above are addressed).
Version 3:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
[Note from the Editor: Reviewer 1 was asked to look over the responses to reviewer 5]

The authors have improved the quality of the manuscript in response to the reviewers' comments. In the self-assembly of triblock polymers, if the spherical domains of end-blocks are randomly distributed in the system, they typically give rise to several broad scattering peaks with a disordered structure. These curves are usually analyzed using the Percus-Yevick hard-sphere model. However, precisely fitting these curves across the entire range of the scattering vector (q) is challenging and often requires consideration of factors such as polydispersity, the radius of the hard sphere, and the volume fraction of hard spheres, as noted by Reviewer 5. In this study, only one characteristic peak was observed, which increases uncertainty in fitting the scattering curve using the Percus-Yevick hard-sphere model. This characteristic peak likely corresponds to the average distance between the spherical domains. The referee agree that the authors remove the SAXS analysis based on the Percus-Yevick hard-sphere model. The current version of the manuscript is now considered acceptable.

REVIEWER COMMENTS

Reviewer #1

General Comment: The work by Elisabeth et al. reported on the porous hydrogel assembly from ABA block polymers, exhibiting a hierarchical structure with an extremely low elastic modulus, strain-hardening behavior and high elasticity with a low hysteresis. The experiments are carefully conducted, and a large amount characterization are performed. The work is nice, while some data require further analysis. The referee recommends publishing this work after a major revision.

Response: *We thank Reviewer #1 for their kind comments.*

Comment 1: Page 4, line 77-84. Sufficient citations should be inserted to support the demonstration of differences between the current work and previous works.

Response 1: *We have added more details and references to demonstrate the differences between current work and previous work. Overall, the submitted manuscript reveals how initial polymer solution conditions for rapid injection processing impact the hierarchical structure and thus dictates the mechanical properties. We undeniably confirm through in situ optical microscopy that the pore deformation significantly contributes to the mechanical response, which has yet to be confirmed in previous hydrogel systems. It is worth noting that a single triblock copolymer at constant molecular weight and block volume fractions is used to access drastically different pore structures and mechanical properties through a straightforward processing approach. The reported hydrogels are distinctly different from all previous works in that the reports either synthesize a library of polymers or use complex processing procedures to achieve the range of mechanical properties. Furthermore, a combination of predicted phase behavior and solvent transport models support the proposed phase separation mechanism, resulting in highly porous hydrogels that exhibit structurally dependent mechanical properties. Finally, the structurally dependent mechanical properties are reminiscent to mechanical metamaterials, yet we use a bottom-up self-assembly approach.*

We have revised the first paragraph of page 4 to highlight these stated differences,

Previous work from our group has described the necessary experimental procedures to produce highly elastic porous hydrogels using rapid injection processing,[1-3] but the effect of microstructure/nanostructure on the mechanical response is unknown. Here, we reveal how initial polymer solution conditions for rapid injection processing impact the hierarchical structure, and thus dictate the mechanical properties, using a single triblock copolymer at constant molecular weight and block volume fraction. The reported hydrogels are distinctly different from all previous works in that the reports either synthesize a library of polymers or use complex processing procedures to achieve the range of mechanical properties.[4] Furthermore, a combination of predicted phase behavior and solvent transport models support the phase separation process, resulting in porous hydrogels that exhibit structurally dependent mechanical properties using a bottom-up approach as opposed to top-down additive manufacturing techniques.[5]

Comment 2: Page 14, line 317-320. Although the authors mentioned that the hydrogels exhibit strain-hardening behavior, no detailed analysis of this behavior was carried out in this manuscript.

Response 2: *The reviewer is correct in that we have not analyzed the strain-hardening behavior. Upon further analysis, we have decided to change the term "strain-hardening" to "non-linear elasticity" regarding the reported hydrogels because the deformation at high elongations (6x the original length) is reversible. As shown in Figure 5c, the reversible deformation and non-linearity of the stress-strain curve*

would characterize these hydrogels as "hyperelastic" (see Melly et al. Int. J. Mech. Syst. Dyn. 2021, 1, 71, DOI: 10.1002/msd2.12013). Therefore, we have replaced "strain hardening" with "non-linear elasticity" when defining the hydrogel mechanical response under uniaxial extension. We have also noted the "hyperelastic" properties of the hydrogels in the Introduction.

Furthermore, we have added more interpretation of the non-linear elasticity behavior at the top of page 13,

Interestingly, the non-linearity stress-strain response in the high-strain region for the samples prepared using THF (**Figure 5a**) is steeper for samples exhibiting smaller pores (i.e., higher initial polymer/THF concentrations) and that the non-linearity stress-strain response occurs at larger strains for samples prepared using DMF as compared to THF (**Figure 5a**). The difference in the onset of strain-stiffening between THF and DMF samples is attributed to the pore size and pore microstructure, highlighting new avenues for controlling mechanical responses in hydrogels through microstructure as opposed to molecular modifications.

Comment 3: Page 14, line 309. The authors stated that the reported porous hydrogels are brittle; however, a referenced work (Chem. Mater. 2022, 34, 10995−11006) demonstrates that the triblock porous hydrogels are highly resilient.

Response 3: *It is generally accepted and shown that adding pores negatively impacts hydrogels, but recent publications, including the Chem. Mater. 2022, 34, 10995 article, indicate that open pores can result in tougher hydrogels. We have revised the sentence on page 14 to now read,*

"…adding pores to hydrogels typically negatively impacts the mechanical properties,[6] although recent results indicate that open pores can enhance the properties of hydrogels (**Figure 5f**).[7-14]"

Comment 4: The corresponding diagrams in SI are necessary to be mentioned in the main text. e.g. Figure S5, S6.

Response 4: *Figure S5 is noted on page 6 and Figure S6 is mentioned on page 12.*

Comment #5: Page 3, line 60-66. The references are necessary to mention at the annotation of Figure 1a because the morphological images (i, ii, iii) were clearly captured from other studies.

Response 5: *The morphological images were not captured from other studies. The morphological images are from the same SOS polymer sample used throughout the manuscript.*

We have added more detail to the figure caption and text to prevent confusion.

On page 2, "The TEM image, cryo-SEM image, and photograph shown in **Figure 1a i – iii** are results from the work described here."

Figure 1 caption,

- "The spherical micellar domains in **Figure 1a i** were characterized via TEM and stained with uranyl acetate."
- "The cryo-SEM image of the porous microstructure shown in **Figure 1a ii** was prepared using a 10.5 wt% polymer solution in DMF."
- "of the prepared hydrogel fiber after rapid injection processing."

Comment 6: Figure 5b There are missing necessary annotation to distinguish what the red and blue symbols represent, respectively.

Response 6: *The red and blue symbols represent hydrogels prepared using THF and DMF, respectively. We have added annotation for completeness.*

Reviewer #3

General Comment: The revised manuscript is suitable to be published.

Response: *Thank you!*

Reviewer #4

General Comment: The authors Lloyd et al. proposed a bottom-up method to produce highly porous hydrogel fibers that resemble extracellular matrices both structurally and mechanically. The underling mechanism for the formation of the microstructure of the hydrogel is said to be the liquid-state spinodal decomposition. My recommendation is that the current work should not be published in Nature Communications.

The critical concerns of the reviewer 2 except comment 4 have not been addressed.
The authors claimed that the comment 3 is beyond the scope of the current work and will do it in future.
For the comment 1, the phase diagram is not improved.

From my experience, the current phase diagram is quite artificial. My specific comments are as follows:

Response: *We strongly disagree with Reviewer #4's comment that we do not address Reviewer #2's comments. Specifically, we have redone the RPA and transport calculations in response to Comment #1 and show that there is a large difference in the spinodal phase boundary between THF and DMF. It is not clear what Reviewer #4 means when they say that the "phase diagram is not improved."*

We would like to point out that Reviewer #2 stated that the reported hydrogels are "a very interesting system that exhibits an intriguing hierarchy of structures and features biomimetic mechanical properties." It is surprising to us that Reviewer #4 has made such a contradictory response.

Comment 1: The authors hypothesize that spinodal decomposition is the underlying mechanism for the microstructure formation. However, this can only be applied for the LIQUID state.
The microstructure observed in experiment is a SOLID structure, at least a soft GEL state rather than a LIQUID structure. In the real case, one has to consider the gelation/solidification in the phase diagram.
A sketch for the transformation of a binary polymer liquid solution into the gel state has been shown in Fig. 12, Hajime Tanaka 2000 J. Phys.: Condens. Matter 12 R207.
The current work focuses on "hydrogel"; it is very empirical when applying a liquid state phase diagram for the formation of hydrogel.

Response 1: *The reviewer has brought up an important issue regarding viscoelastic phase separation. Like Reviewer 2, Reviewer 4 is concerned that the effects of elasticity due to the micellar network are not*

*accounted for in our phase separation mechanism. We acknowledge that elasticity is not accounted for in the model, and we agree with both reviewers that these effects are important for (i) predicting the pore size and (ii) understanding the detailed phase separation kinetics. However, we make no claims to do either (i) or (ii) in the paper. Instead, we simply claim that the **key driving force** for pore formation is a macrophase separation between the SOS polymer and water. Elasticity stabilizes phase separation processes and retards phase separation kinetics. Thus, the RPA calculations in the paper represent conservative predictions of the outer boundaries of the compositions that can drive pore formation. Furthermore, unlike the characterization by the reviewer, we claim that this is not a controversial position viz-a-viz the literature. The figure referred to by the reviewer (reproduced from Fig. 4, Tanaka, Phys Rev E., Vol 59, 1999, p. 6849) is used by Tanaka to discuss how the formation of "transient gels" during phase separation can alter the phase separation kinetics from a spinodal decomposition mechanism to a nucleation and growth-type mechanism. However, in Tanaka's theory **the same driving forces that drive a liquid-liquid phase separation are responsible for the phase separation of the gel**. Thus, the theories of viscoelastic phase separation by Tanaka are completely consistent with our claim that the macrophase separation is the primary driving force and the effects of elasticity are secondary. This is also broadly consistent with our wider reading of the NIPS literature, with which we have 10 years of experience.*

Finally, the reviewer claims that the current theory is "very empirical" with the implication that adding an elasticity term would remedy the situation. Although the models used here and by others (including those by Tanaka) are highly phenomenological, the reviewer mischaracterizes the situation in two key respects. First, it is not trivial to extend Tanaka's treatment of viscoelastic phase separation to include block copolymer thermodynamics because (unlike Tanaka's approach which focuses on colloidal gels), there is a microphase separation endogenous to the theory that is responsible for the elasticity. Thus, it is reasonable for us to defer the creation of a new theory of viscoelastic phase separation for block copolymer materials to a separate publication. Second, because all these types of theories are phenomenological, they are necessarily "empirical". Consequently, there are in fact very few examples in the literature quantitatively comparing the predictions of a parameterized model of polymer phase separation kinetics to experiments. As such, one of the contributions of the present work is a careful parameterization that enables one to begin to make more quantitative comparisons.

We have added a sentence at the top of page 11 to mention viscoelastic phase separation.

Further, this model does not account for the effects of the elasticity of the micellar network, which could shift the spinodals (e.g., the blue boundary separating the microphase ($\mu\phi$) and macrophase (2ϕ) regions) and give rise to more complex viscoelastic phase separation kinetics inside the two-phase region.[15,16] Additional development of more complex models that account for emergent elasticity and detailed numerical simulations are necessary to capture these phenomena.[17] Instead, a more detailed model is needed to capture these phenomena.

Comment 2: Even for the liquid-state phase diagram, there is a big problem. I guess the blue line in Fig.4 is the spinodal line; the authors should clearly indicate the meaning of different lines in Fig.4.
By using the degree of polymerization Np=4436, it is impossible to produce the binodal line according to the Flory-Huggins theory, while the spinodal line can be estimated analytically.

***Response 2:** The blue lines in **Figure 4** are spinodal lines. One line (purple) is the microphase spinodal between the PS and PEO blocks, the other line (blue) is the macrophase spinodal between the triblock copolymer and water. This is already indicated in the main text and in the caption to Figure 4**. However, we have added the statement,* "The blue line in the ternary phase diagrams separating the μφ and 2φ regions represent the spinodal line." *to minimize confusion.*

The reviewer's concern related to Flory-Huggins theory is puzzling. Flory-Huggins theory is a mean-field theory that is valid for homopolymer mixtures and cannot be used to treat the thermodynamics of triblock copolymer solutions. We used a random phase approximation (RPA) to the field theoretic free energy for a triblock copolymer mixture with a good solvent and a poor solvent to calculate the given spinodals (see e.g., Fredrickson, "The Equilibrium Theory of Inhomogeneous Polymers", 2006, Oxford). Binodals can be obtained using e.g., self-consistent field theory or a density functional theory (e.g., Uneyama and Doi, Macromolecules, 38, 196-205, 2005), but we have not done so here.

Contra the unsupported assertion stated by the reviewer, a large degree of polymerization (Np = 4436) does not present a theoretical impediment to obtaining a binodal, but it could make it numerically intractable. In the case of FH theory, a large degree of polymerization will give a branch of the binodal that (for all intents and purposes) has zero polymer concentration. However, we cannot say a priori what the macrophase binodal will look like for the present system. Again, this is because the thermodynamics of the triblock copolymer are different than for a homopolymer.

Comment 3: When producing the liquid phase diagram, the Flory parameter χ should be paid attention carefully. For me, the current parameters χ are arbitrarily selected without too much physical meanings.

Response 3: *As described in the Supplemental Material, the Flory x parameters used in the manuscript were not arbitrarily selected but were obtained from the literature or computed using Hansen solubility parameters when no literature values were available. References for these values, including references for computing them via solubility parameters, are provided in the Supplemental Material.*

We have clarified this point in the main text and added additional references to the Supplemental Material.

*Specifically, near the top of page 10 we have added the sentence, "*The degree of polymerization and block fractions for the model were obtained directly from experiment, and the Flory–Huggins χ-parameters were obtained from literature (where available) or by Hansen solubility parameters. Additional details, including specific references for the χ-parameters, are available in the Supporting Information.*"*

Comment 4: I have also looked at the comments of reviewer 1 and the response letter of the authors.

I agree with reviewer 1 that designing highly extendable, reversibly deformable, and extremely soft hierarchical hydrogels has already been published in the previous work by Prof. Hickey, see Nature Communications 10, 3855, 2019. I have compared the current manuscript with the previous one; the characterization method is quite similar. Thus, the novelty of the current work is limited.

Response 4: *In response to this comment, we would like to reiterate here our response to Reviewer #1's comment:*

The 2019 Nature Commun. article reports the experimental methods for producing hydrogels with hierarchical structures with interesting properties, but the connection between structure and property was unknown. The submitted manuscript uncovers the relationship between hierarchical structure and materials properties and reveals new biomaterial design rules for mimicking both the structure and function of natural tissues. We have revised the introduction to differentiate the current work from previous publications.

We had revised the manuscript to address these concerns. Furthermore, please see Response 1 to Review #1 above for additional discussion regarding the novelty of the work.

Comment 5: In all, I feel that the mechanism for the micro-structure formation of hydrogel presented by the authors is quite artificial. The present manuscript with some revision may be suited for other journals, like Macromolecules.

Response 5: *We fully disagree with the statement that the self-assembly mechanism is artificial and is suited for a different journal. We have combined the predicted ternary phase behavior and solvent transport to support the proposed phase separation mechanism, which is a new way to create hierarchically ordered hydrogels with exceptional properties. Furthermore, Reviewer #1 in the General Comment stated that , "The experiments are carefully conducted, and a large amount characterization are performed." and these experiments support the proposed self-assembly mechanism.*

References

1 Lang, C., Kumar, M. & Hickey, R. J. Influence of block sequence on the colloidal self-assembly of poly(norbornene)-block-poly(ethylene oxide) amphiphilic block polymers using rapid injection processing. *Polym. Chem.* **11**, 375-384 (2020). https://doi.org:10.1039/C9PY00954J

2 Lang, C. *et al.* Solvent-Non-Solvent Rapid-Injection for Preparing Nanostructured Materials from Micelles to Hydrogels. *Nat. Commun.* **10**, 3855 (2019). https://doi.org:10.1038/s41467-019-11804-7

3 Lang, C. *et al.* Nanostructured block copolymer muscles. *Nat. Nanotechnol.* **17**, 752-758 (2022). https://doi.org:10.1038/s41565-022-01133-0

4 Foudazi, R., Zowada, R., Manas-Zloczower, I. & Feke, D. L. Porous Hydrogels: Present Challenges and Future Opportunities. *Langmuir* **39**, 2092-2111 (2023). https://doi.org:10.1021/acs.langmuir.2c02253

5 Jiao, P., Mueller, J., Raney, J. R., Zheng, X. & Alavi, A. H. Mechanical metamaterials and beyond. *Nat. Commun.* **14**, 6004 (2023). https://doi.org:10.1038/s41467-023-41679-8

6 LaNasa, S. M., Hoffecker, I. T. & Bryant, S. J. Presence of pores and hydrogel composition influence tensile properties of scaffolds fabricated from well-defined sphere templates. *J. Biomed. Mater. Res. Part B* **96B**, 294-302 (2011). https://doi.org:https://doi.org/10.1002/jbm.b.31765

7 Sano, K., Ishida, Y. & Aida, T. Synthesis of Anisotropic Hydrogels and Their Applications. *Angew. Chem. Int. Ed.* **57**, 2532-2543 (2018). https://doi.org:https://doi.org/10.1002/anie.201708196

8 Han, L. *et al.* Tough, self-healable and tissue-adhesive hydrogel with tunable multifunctionality. *NPG Asia Materials* **9**, e372-e372 (2017). https://doi.org:10.1038/am.2017.33

9 You, F., Wu, X. & Chen, X. 3D printing of porous alginate/gelatin hydrogel scaffolds and their mechanical property characterization. *Int. J. Polym. Mater. Polym.* **66**, 299-306 (2017). https://doi.org:10.1080/00914037.2016.1201830

10 Song, X. *et al.* A Novel Human-Like Collagen Hydrogel Scaffold with Porous Structure and Sponge-Like Properties. *Polymers* **9** (2017). https://doi.org:10.3390/polym9120638

11 Luo, R., Wu, J., Dinh, N.-D. & Chen, C.-H. Gradient Porous Elastic Hydrogels with Shape-Memory Property and Anisotropic Responses for Programmable Locomotion. *Adv. Funct. Mater.* **25**, 7272-7279 (2015). https://doi.org:https://doi.org/10.1002/adfm.201503434

12 Lu, M. *et al.* Phase-Separation-Induced Porous Hydrogels from Amphiphilic Triblock Copolymer with High Permeability and Mechanical Strength. *Chem. Mater.* **34**, 10995-11006 (2022). https://doi.org:10.1021/acs.chemmater.2c03004

13 Baur, E., Hirsch, M. & Amstad, E. Porous 3D Printable Hydrogels. *Advanced Materials Technologies* **8**, 2201763 (2023). https://doi.org:https://doi.org/10.1002/admt.202201763

14 Alsaid, Y. *et al.* Tunable Sponge-Like Hierarchically Porous Hydrogels with Simultaneously Enhanced Diffusivity and Mechanical Properties. *Adv. Mater.* **33**, 2008235 (2021). https://doi.org:https://doi.org/10.1002/adma.202008235

15 Hajime, T. Viscoelastic phase separation. *J. Phys. Condens. Matter* **12**, R207 (2000). https://doi.org:10.1088/0953-8984/12/15/201
16 Tanaka, H. Viscoelastic model of phase separation in colloidal suspensions and emulsions. *Phys. Rev. E* **59**, 6842-6852 (1999). https://doi.org:10.1103/PhysRevE.59.6842
17 Ajji, A. & Choplin, L. Rheology and dynamics near phase separation in a polymer blend: model and scaling analysis. *Macromolecules* **24**, 5221-5223 (1991). https://doi.org:10.1021/ma00018a031

Response to Reviewers

Reviewer #1

Comment: I have read the revised manuscript, and the authors have already addressed the referees' comments, as well as answered Reviewer 4's comments. I recommend that this work be published in this journal.

Response: *We thank Reviewer #1 for their comments.*

Reviewer #4

Comment 1: I am not convinced that the very simplified mechanism (Figure 4) proposed by the author is sufficient to address the formation of hydrogels. From my opinion, this is a hand-waving theory which cannot be verified in physical modelling and is far from real physics of hydrogels.

Response 1: *We have responded to this criticism extensively in our previous reply. There we stated that: "We acknowledge that elasticity is not accounted for in the model, and we agree … that [it is] important for (i) predicting the pore size and (ii) understanding the detailed phase separation kinetics. However, we make no claims to do either (i) or (ii) in the paper. Instead, we claim that the **key driving force** for pore formation is a macrophase separation between the SOS polymer and water. Elasticity stabilizes phase separation processes and retards phase separation kinetics. Thus, the RPA calculations in the paper represent conservative predictions of the outer boundaries of the compositions that can drive pore formation."*

Furthermore, even though we are not making claims about the detailed phase separation kinetics, the predictions of the spinodals using the random phase approximation and the mass transport model are significantly more sophisticated than "hand-waving." Both Reviewer #1 and Reviewer #3 support this assertion and state that the paper should be published. Indeed, Reviewer #1 specifically states that we have responded to Reviewer #4's comments.

Comment 2: With such a high degree of polymerization, 4436, in the Flory-Huggins theory, as presented by the authors, it is almost impossible to capture the spinodal decomposition in a physical modeling, for example, the Cahn-Hilliard model. The problem is that due to the large degree of polymerization, the polymer-rich phase and the polymer-lean phase reach the binodal line asynchronously; the binodal line is not shown by the authors in Figure 4. Suffice to say, when the polymer-lean phase reaches the binodal line, the polymer-rich phase still needs a much longer time to arrive at the binodal line due to the distinct entropy contributions of polymer and solvent arising from the high degree of polymerization. If only considering the liquid-state phase separation, as proposed by the authors, the mass is not conserved any more for such a high degree of polymerization. Usually, a very small value for the degree of polymerization (for example, 5, 10, 20, some values like this) is adopted in the simulation to capture the spinodal decomposition (see J. Chem. Phys. 158, 214903 (2023) and similar works).

Response 2: *We agree with the reviewer's characterization of the phase separation *kinetics* that are described here. For example, during the phase separation process, the emergence of the polymer-rich phase will be retarded by gelation. As the reviewer states, this means the polymer-lean phase will reach the binodal asynchronously from the time that the polymer-rich phase reaches its binodal. As a matter of fact, one of the co-authors of this manuscript (DRT) co-authored the paper referenced [Garcia, DRT, et al. J Chem Phys 158, 214903 (2023)] that details how asymmetric dynamics can lead to such behavior even without the presence of elasticity in the model.*

Because we were heavily involved on the referenced paper (DRT wrote the simulation software, generated data, participated in training the first author, and advised other student co-authors), we find it confusing that the reviewer is quoting Garcia et al. to us as an argument from authority that the present contribution is incorrect. More importantly, the principle behind the argument is also incorrect. Garcia et al. does use a small degree of polymerization in the simulations (N=20), but this choice is justified by numerical considerations (simulation stability and a desire to limit the range of timescales), rather than a fundamental impossibility of spinodal decomposition at large N. Indeed, if spinodal decomposition was impossible at a large degree of polymerization, which it clearly is not, then experimental evidence for this phenomenon would be widespread. Additionally, the results of the RPA model are relatively insensitive to the degree of polymerization when N is large, so the specific choice of this parameter is largely unimportant.

*As we stated above (and in previous replies), we suppose that the reviewer is concerned about our treatment of *kinetics*, when instead we are claiming that the RPA model in the present manuscript contributes an *equilibrium* phase diagram that provides information about the *driving forces*. These driving forces have implications for the kinetics, but we stop well short of making claims of the details of these kinetics. Indeed, we are very careful to avoid doing so.*

Comment 3: One possible way to amend this is to add the gelation mechanism in the theory, as suggested in my previous report. The authors have to address this mechanism before publication.

Response 3: *The request by the reviewer to add a gelation mechanism represents a major new contribution that is both unnecessary and justifiably well beyond the scope of the present paper. We dispute the claim that the present RPA spinodals do not provide relevant information about the phase separation. Additionally, in our opinion adding gelation to the present theory would contain enough significant results to justify an entire paper for the theory alone. To fully explain why this is so, we need to refer to the literature and provide additional technical details.*

The gelation mechanism referenced by the reviewer in the previous reply references work by Tanaka (Phys Rev E, 59, 6842-6852, 1999, doi:10.1103/PhysRevE.59.6842 and Phys Rev E, 56, 4451-4462, 1997, doi: 10.1103/PhysRevE.56.4451) on the phase separation behavior of colloidal gels. The free energy functional in this case is given by

$$F = \int dr \left[f_{mix}(\phi) + \frac{C}{2}(\nabla\phi)^2 + \boldsymbol{\sigma}:\boldsymbol{\epsilon} \right] \qquad (1)$$

where ϕ is the volume fraction of colloids, $f(\phi)$ is a homogeneous free energy of mixing, C is a coefficient for the term penalizing gradients (the term gives an interfacial free energy) and $\boldsymbol{\sigma}$ and $\boldsymbol{\epsilon}$ are stress and strain tensors (defined in such a way as to be thermodynamically conjugate).

*Notably there is an *asymmetric stress division* between the colloidal network and the solvent, so the stress in this case is only applied to the colloidal network. This is important because the solvent cannot sustain an elastic stress on the relevant timescales. In other words, because this system has only two phases (a colloidal network phase and a solvent phase) there are simplifying assumptions that the authors must make about how the phenomenological parameters in the constitutive model depend on ϕ for the theory to be even qualitatively valid.*

Given the explicit expression for the free energy functional it is possible to compute spinodals using the second functional derivative of Eq (1),

$$\frac{\delta^2 F}{\delta \phi^2} = 0. \qquad (2)$$

This functional derivative will involve a familiar first term (the typical homogeneous spinodal $\partial^2 f/\partial\phi^2 = 0$) and something from the third term related to the elasticity (depending on the functional dependence of the stress and strain to the volume fraction of colloids, ϕ).

This approach has been extended beyond colloidal systems, and recently (2021) Yoshimoto and Taniguchi (J Chem Phys, 154, 104903, doi:10.1063/5.0039208) published a nice paper discussing nonsolvent-induced phase separation (NIPS) with elasticity for a ternary system consisting of a homopolymer, solvent, and nonsolvent. Here the free energy involves more terms, due to the ternary nature of the system, but it still consists of the same three basic quantities,

$$F = \int dr\, [\, f_{mix} + f_{int} + f_{ela}\,] \qquad (3)$$

where f_{mix} is the mixing free energy density, f_{int} is the interfacial free energy density, and f_{ela} is the elastic free energy density.

We highlight the paper by Yoshimoto and Taniguchi (YT) for two important reasons.

1. *This paper is a demonstration of how much work is required to add a gelation mechanism. There is an entire paper (c. 2021) dedicated to studying the effects of adding elasticity to a ternary NIPS model. This builds on previous work by others (e.g., DRT et al, Soft Matter, 13, 3013, 2017, doi: 10.1039/C6SM02839J) of ternary homopolymer NIPS without elasticity.*
2. *This paper highlights the value of using a spinodal from the free energy of mixing (i.e., $\partial^2 f_{mix}/\partial\phi^2 = 0$) that neglect the elasticity (Fig. 2 in YT). This spinodal curve is the analogue of the RPA spinodals in our manuscript. Indeed, the use of such a curve is common in the NIPS literature.*

In the case we consider in this manuscript (an ABA triblock copolymer solution), it is not possible to write the non-elastic free energy as an explicit function of the volume fraction without additional approximations. Using a weak inhomogeneity expansion gives

$$F[\varphi_i(\boldsymbol{r})] = F[\bar{\varphi}_i] + \frac{k_B T}{2}\sum_i^{n_s}\sum_j^{n_s}\frac{1}{(2\pi)^d}\int d\boldsymbol{q}\, \hat{\Gamma}_{ij}(\boldsymbol{q})\hat{\psi}_i(\boldsymbol{q})\hat{\psi}_j(-\boldsymbol{q}) + \cdots \qquad (4)$$

where the important quantity is the second vertex function $\hat{\Gamma}_{ij}(\boldsymbol{q})$. We provide extensive details in the SI about how the RPA approximation is used to calculate the second vertex function and subsequently obtain spinodals.

There are two technical challenges that need to be overcome in order to follow the formalism previously used by Tanaka and YT to add elasticity to Eq. (4) for an ABA triblock solution.

1. *One needs an explicit expression for the free energy that is valid beyond the weak segregation limit. This can in principle be done using the formalism developed by Uneyama and Doi (Macromolecules, 38, 196-205, doi:10.1021/ma049385m), but this step is not trivial.*
2. *One needs to create and parameterize an appropriate constitutive model for triblock copolymers that undergo both microphase and macrophase separation. This is a challenging task that has not been done in the literature (to the best of our knowledge). Note that the development of such a model goes well beyond the work of Tanaka and YT because there are now *two* phases that can sustain a stress (A-blocks that microphase separate into micelles and ABA polymers that macrophase separate). Both of these phases have to be accounted for in the constitutive model and will need to have parameters with an appropriate composition dependence to be meaningful. Note that there is some prior work developing such models for a microphase separated AB diblock melt by Yoo and Vinals (Macromolecules, 45, 4848-4856, 2012, 10.1021/ma300547y) showing just how difficult this could be.*

*Finally, we highlight two recent papers by the Fredrickson group that are also highly relevant (Grzetic et al., ACS Macro Lett, 12, 8-13, 2022, doi: 10.1021/acsmacrolett.2c00611 and Cooper et al., J Chem Phys., 160, 074903, 2024, doi: 10.1063/5.0188196). In these papers Fredrickson et al. use dynamic SCFT to simulate self-assembly and nonsolvent-induced phase separation (SNIPS) for an *AB diblock copolymer* mixed with a solvent and nonsolvent. As part of these papers, they employ a methodologically identical RPA expansion to compute spinodals, which they use to analyze their simulations of the phase separation kinetics. These simulations contain no explicit elasticity, but they include a slow mobility for the B-block that mimics glassy dynamics. However, *these slow kinetics are not included in the spinodals*. Again, this demonstrates that RPA computed spinodals are deeply informative for NIPS-like processes even without explicitly accounting for a gelation mechanism.*

*In summary, the request by the reviewer to add a gelation mechanism is both *unnecessary* and *well beyond the scope* of the present work. We have cited recent papers that neglect elasticity in the computation of spinodals to perform a similar type of analysis to what we have done here. Furthermore, we have discussed how elasticity would be added to our model and demonstrated that the requested task represents a significant amount of new research. As such, we strongly disagree with the reviewer that such a step is needed for the current paper to be publishable.*

Comment 4: The current mechanism in Figure 4 is far from real physics and is too simple with only considering the liquid phase diagram. This simplified theory is at the time stage of 1940s and is not the-state-of-the-art.

***Response 4:** The claim that our model sophistication is appropriate for the time-period circa 1940 is a gross mischaracterization of our work. The random phase approximation (RPA) that we used for computing the spinodal curves was not discovered or used in polymer physics until the late 1970s and early 1980s. For example, Scaling Concepts in Polymer Physics by P.G. de Gennes was published in 1979 and L. Leibler's seminal paper "Theory of Microphase Separation in Block Copolymers" which applied the RPA to a diblock was published in 1980 (Macromolecules, 13, 1602-1617, doi: 10.1021/ma60078a047). Fredrickson and Leibler were among the first to publish RPA results for block copolymer solutions in 1989 (Macromolecules, 22, 1238-1250, doi:10.1021/ma00193a040), Werner and Fredrickson worked out the RPA for a triblock copolymer melt in 1997 (J. Polymer Sci B, 35, 849-864, doi:10.1002/(SICI)1099-0488(19970415)35:5<C849::AID-POLB14>3.0.CO;2-A), and Uneyama and Doi worked out the general case for N-blocks and M-components in 2005 (Macromolecules, 38, 196-205, doi:10.1021/ma049385m). The latter was a key reference to our five-component system (Blocks A, B, C, solvent, nonsolvent). Furthermore, the computation of the spinodals required the creation of a custom Python code that relies heavily on numerical methods, including linear algebra (inversion of 5x5 component matrices) and the solution to nonlinear equations (zeros of the RPA equation) for *each point on the spinodal*. As such, the computation of this RPA solution is very much state-of-the-art. Indeed, Reviewer #2 pointed out in a prior response that a comparable computation of spinodals was performed by Grzetic et al. in ACS Macro Letters in 2023 (vol. 12, pp. 8-13, doi:10.1021/acsmacrolett.2c00611) though this latter system has only four components (an A-B diblock with solvent and nonsolvent).*

In addition, while the mass-transfer model is not as sophisticated as the RPA model, there are much more recent literature precedents than the 1940s. Tsay and McHugh published a 1D mass transfer model of the NIPS processes in 1990 (J. Polymer Sci B, 28, 1327-1365, doi:10.1002/polb.1990.090280810) and of one with a moving boundary in 1991 (Chem. Eng. Sci., 46, 1179-1187, doi:10.1016/0009-2509(91)85111-A). In addition, DRT and co-authors recently (2019) published a paper on the coupling between diffusive mass-transfer and phase separation kinetics (Soft Matter, 15, 4614-4628, doi:10.1039/C9SM00355J)

As discussed in detail above, our model is very much in line with recent contributions to the literature on the phase separation of block polymer solutions. Our novel contributions in this paper include a calculation

*of RPA spinodals for a more complex NIPS system than has been done to date (5-components: 3 polymer blocks, solvent, nonsolvent), a spatially averaged mass transfer model specifically designed to include hydrogel swelling, and critically, *a careful parameterization of both the RPA and transport models to experimental data*. The latter is a highly nontrivial contribution that is largely absent from the literature. The phase field/DSCFT/DDFT models that characterize this field contain numerous phenomenological parameters that make comparison to experiment difficult. Overcoming this challenge has required a strong collaborative connection and significant work, and it represents a meaningful contribution to the literature.*

Comment 5: The authors should also improve the writing style and present the figures in a more clear way. For example, in the caption of Figure 4, "a)...., the lines show the predicted..", which lines? there are several lines, light blue, dark blue, cyan? Such a presentation style is very ambiguous.

Response 5: *We have updated the caption of Figure 4 to now read,*

"The bold lines, as indicated in the legends on the top right of each ternary phase diagram, show the predicted average solvent/water/polymer concentrations of the fiber as a function of time ($t = 0$ to 5 s) from the model of solvent exchange. b) The ternary phase diagram of water, DMF, and SOS with superimposed solvent exchange model prediction at the same concentrations used for the samples. The light blue line in the ternary phase diagrams separating the $\mu\phi$ and 2ϕ regions represent the spinodal line."

Response to Reviewers

Reviewer #4

Comment: I am grateful to the authors for their efforts in writing the rebuttal letter. However, there is no improvement of the phase diagram in Figure 4 at all. I mean for the large degree of polymerization used by the authors, the BINODAL line is impossible, not the spinodal line argued by the authors. The current phase diagram is nothing related to hydrogel. It is a kind of artificial phase diagram without physical meanings. It has only arbitrary spinodal lines which cannot be verified in the experiments. The binodal line is missing, when the authors emphasize on the importance of the equilibrium rather than the kinetics. Experiments should be performed to confirm the binodal as well as the spinodal lines. In all, the current manuscript has serious flaws in the theory and is not suited for Nature Communications.

Response 1: *We understand that the current predicted phase diagrams represent a simplified version of the complicated phase behavior of the reported hydrogels. The proposed theory is an attempt to support the hypothesis of a two-step self-assembly process (i.e., nanoscale self-assembly and macrophase separation). It is clear that we disagree about the validity of the proposed theory and are at an impasse. Therefore, to overcome the continued gridlock, we have decided to remove Figure 4 and the theory portion from the manuscript. Figure 4 and the content associated with the figure will be revised and used in a different manuscript.*

We would like to point out that the hydrogel structure dependent properties are what is exciting and novel in synthetic materials. There has yet to be a hydrogel system with the combination of properties as demonstrated in the manuscript. Furthermore, we reveal that the hierarchical structure spans many orders of magnitude through bottom-up methods, leading to new and exciting hydrogel processing methods.

Changes: We have removed Figure 4 and the text in the manuscript and supporting information related to the predicted phase diagrams. We have added text to support the proposed two-step self-assembly process where micelles first form and then microscale pores, resulting in the hierarchical structure. The revised text is below in yellow.

The exact reason for the microstructural differences between hydrogel fibers prepared with THF and DMF is still under debate. We hypothesize that at the initial stages of solvent exchange PS micelles form due to water infiltration into the fiber. Figure 2a shows the formation of micelles at lower initial polymer solution concentrations. The mechanism of micrometer sized pore formation in the reported hydrogels is posited to be analogous to macropore formation in separation membranes prepared using nonsolvent-induced phase separation (NIPS). The reason for the stark differences in microstructural morphology between fibers prepared using DMF and THF is not immediately apparent and is being actively explored. There is a general rule of thumb in the NIPS community that polymer solution viscosity and solvent exchange rates control structure, but both parameters are nearly identical under these experimental conditions (Figure S2 and S17), and neither parameter holds explanatory power. Thus, microstructural variations are expected to be due to changes in the ternary phase diagrams (i.e., polymer, solvent, and water) and solvent exchange trajectories where the compositional pathways during solvent exchange traverse phase boundaries at different times.

Reviewer #5

Comment 1: In my review of the revised manuscript I also have concerns about the applicability of the simplified model (as Rev. 4). The mechanism of macrophase separation between PS-PEO-PS and water is not well understood.

Response 1: *Due to the disagreement regarding the predicted phase diagrams and inability to come to a consensus, we have removed Figure 4 and the text in the manuscript and supporting information related to the predicted phase diagrams. The specific details related to the phase separation process that results in hierarchically ordered hydrogels are under debate, but we would like to state that the experimental results clearly show that the hierarchical structure of the hydrogels directly impact the mechanical response. The combination of SAXS, SALS, and confocal microscopy of the hydrated fibers confirm the impact of the hierarchical structure on the mechanical properties. We have softened the text regarding the proposed hydrogel formation mechanism. Please see Response 1 to Reviewer #4 for more detail.*

Comment 2: In addition to this, I find the explanation of the scattering data not consistent with the applied model (interactions between spheres described by a Percus-Yevick hard sphere interaction potential). Specifically, in the SI the authors provide some fits to the model (Figure S4) and discuss the parameters (domain spacing, micelle diameter and volume fraction) in Figure S5. However, the PY model provides the domain spacing (from the maximum of the structure factor) which is ~ 80 nm as discussed, the sphere radius (from the first minimum of the form factor, at qR=4.493) and the hard sphere radius. In addition, it provides the volume fraction of spheres and requires some polydisprersity. The problem is that the reported values do not match with the experiment. For example, the first minimum in the form factor is at ~ 0.1 nm-1 giving a sphere radius of ~45 nm, i.e. much larger than the reported values. In addition, the hard sphere radius is not clearly reported. Lastly, polydispersity is not applied, although clearly needed (minima are not clearly seen). One related point: the model does not fit the whole scattering curve – but a part of it (Figure S4). What is the reason?

Response 2: *The comments regarding the SAXS fitting are insightful and valid, and we have revised the text. First, we had difficulty in fitting the full q-range due to the significant low-q scattering and smeared spherical form factor oscillations at higher-q. Therefore, the reported values from the PY model deviate from direct analysis of the 1D patterns, as noted by the reviewer. We know confidently that the domain spacing (i.e., center-to-center micelle distance) is ~80 nm, which was calculated from the primary scattering peak. The PS micelle core diameter (~20 nm) was calculated from TEM analysis (see Figure 2a). Therefore, we have removed the SAXS model analysis due to the limited q-range. The revised text is shown below in yellow.*

Synchrotron SAXS measurements confirmed the disordered organization of the micelle network in the hydrogels. The domain spacing d of the micelle network was determined from the one-dimensional (1D) scattering intensity versus scattering vector (q) shown in Figure 2b using the relationship $d = 2\pi/q^*$, where q^* is the primary scattering peak associated with the center-to-center micelle distance. The average center-to-center micelle distance was calculated to be 80 nm. In-depth SAXS analysis was conducted using a spherical form factor and Percus-Yevick hard sphere approximation structure, but fitting the full q-range was challenging due to the significant low-q scattering and smeared spherical form factor oscillations at higher-q. The low-q upturn is indictive of hydrogel heterogeneity and larger scale features, which is consistent with the hierarchical structure. Therefore, the SAXS patterns were interpreted by tracking the change in q^*. Variations in domain spacing with solvent and concentration are evident in the change of q^* but are within a 5 nm range. The consistency in the domain spacing, despite variations in the viscosity and solvent, confirms the nanostructure is insensitive to the sample conditions used in the study.

Comment 3: Another point relates to the stress-strain data. The stress-strain data of Figure 5 show strain hardening. It is mentioned that the difference in the onset of strain hardening is due to the pore size and pore microstructure…highlighting new avenues for controlling mechanical responses..". It is not clear how the two are related.

Response 3: *The porous structure of cellular solids is known to play a critical role in the mechanical properties of natural materials (see "Cellular Solids" by Gibson and Ashby, Cambridge University Press, 2nd Ed. 1997). Pore size and shape have a direct influence on the mechanical response of materials and is nicely demonstrated in the work shown in the manuscript. Specifically, we show that as the micrometer sized pores increase, the modulus decreases, which is consistent with natural cellular solids. The reported strain hardening is attributed to PEO chain stretching at high strains. As described in the manuscript,*

"the total strain is unevenly portioned between the pores and the bridging PEO chains. The initial pore deformation requires very little force, which is insufficient to cause chain extension. Chain extension therefore occurs at larger strains, which is expected to result in non-linear elasticity behavior at large strains."

The reason for the differences in the onset of strain hardening is that smaller pores will maximally deform at lower strains, resulting in an earlier onset of strain hardening due to the stretching of the PEO chains.

Comment 4: A lesser point: ref [35] is in error.

Response 4: *We have removed the discussion regarding the predicted phase diagram and therefore have removed reference 35.*

Comment 5: For the above reasons and the comments of Rev.4 I do not feel that the work can be published by Nature Communications. I could suggest, instead, a more specialized journal (after the points above are addressed).

Response 5: *The hydrogel structure dependent properties are exciting and novel and have yet to be implemented in synthetic materials. There has yet to be a reported hydrogel system with the combination of properties as demonstrated in the manuscript. Therefore, the Nature Communications is a perfect journal to publish the manuscript.*