The authors would like to thank the reviewers for their valuable time and effort in evaluating our manuscript. The following constitute our responses (**shown in bold**) to the reviewers’ comments. Revisions within the manuscript are highlighted as well.

**Referee 1**

Major Concerns:

The advantage of this method is not fully addressed. Is high yield of ECM by this method compared to the other methods? Dose the ECM produced by this method function in vivo? The short abstract (summary) should be rewritten more precisely what they did, and the discussion part should be more related to the results we present.

**The summary has been edited for clarity while staying within the 50-word maximum.**

**Additional detail has been added to the representative results and discussion sections, particularly regarding the use of the hollow fiber casting system, as well as specific references to figure panels not previously specifically referenced in the text.**

Minor Concerns:

1. The all abbreviations should be clear indicated (for example: Line 317: DI, line 323: SEM; Line 325 HFM?)

**“DI” at line 317 has been clarified to “deionized water”**

**“SEM” at line 323 has been clarified to “scanning electron microscopy”**

**“Title of figure 3 has been clarified to “cell viability on hollow fiber membranes”**

2. Line 121: how did they calculate "17.8 w/w%", should be "22.3% w/w"?

**The mass of Polysulfone to be used has been corrected to 70 g in section 1.1 of “Production of extracellular matrix using sacrificial hollow fiber membranes”**

**The volume of NMP to be used for the polymer solution has been corrected to 314 mL (323.3g, density NMP = 1.03 g/mL) in section 1.2 of “Production of extracellular matrix using sacrificial hollow fiber membranes”**

**w/w% = (weight solute/weight solution)\*100**

**w/w% = (70g/(70+323.3g))\*100 = 17.8 w/w%**

3. Line 204: what is container used for solution?

**“a sterile 1.5 mL microcentrifuge tube” has been specified in section 4.1 of “Production of extracellular matrix using sacrificial hollow fiber membranes” for preparing the 1 mg/mL stock solution of bovine plasma fibronectin in PBS.**

**“a sterile 50 mL conical centrifuge tube” has been specified in section 4.1 for preparing the diluted solution of bovine plasma fibronectin in PBS (50 mL)**

**pH of sterile PBS to be used has also been specified in section 4.1**

4. Line 245: "3." Should be "2."?

**The section header number for “Decellularization of extracellular matrix” has been corrected from “3.” to “2.”**

5. Line 257: this is not a step, should label as a note

**The step previously labeled as 1.3.2 has been relabeled as a note.**

6. Line 271-274: How to prepare Dnase 50KU? KU is KiloUnit?

**Units for preparation of DNAse I and RNAse A in section 2.1.5 of “Decellularization of extracellular matrix” have been clarified. U (enzyme unit, kU => kilounits) refers to the amount of an enzyme necessary to catalyze the reaction of 1 micromole of substrate per minute.**

7. in Figure 3, a fibroblast cell marker should be stained and present.

**The authors recognize that evidence of fibroblast phenotype could be helpful and point to the original research publication where several lines of fibroblasts were used.**

**Roberts, K., Schluns, J., Walker, A., Jones, J. D., Quinn, K. P., Hestekin, J., & Wolchok, J. C.. Cell derived extracellular matrix fibers synthesized using sacrificial hollow fiber membranes. *Biomedical Materials*, *13*(1), (2017)**

**Referee 2**:

Minor Concerns:

lines 198- 260 are highlighted and I was not sure of the purpose.

**Sections were highlighted in yellow which the authors judged to be of interest for portrayal in the JoVE methods video as directed in the JoVE Instructions for Authors.**

Reviewer #3:

Major Concerns:  
none  
  
Minor Concerns:  
(1) In addition to its therapeutic significance, this high-yield ECM production method can be of interest to researchers working in biomechanics, cellular migration (e.g. durotaxis) or biosensing given the recent findings on ECM-bound nanovesicles, the exosome-like cargo-carrying biomarker particles which are now identified to be an integral and functional key component of the ECM. Given the strong medical/clinical significance it is reasonable that the authors put the weight for the motivation of the method to these areas, but it would broaden the perspective of the reader if a few sentences are included pointing to the research fields for which the method can be useful for, other than biomedicine.

**The authors recognize the potential broader utility of this process and have added relevant commentary in the discussion section.**

(2) It is not clear if all required elements of the HFM Spinneret from AEI are commercially available. In the earlier research articles by the authors (Biomedical Materials 2018), the Spinneret from the same vendor, seem to contain custom-built parts. This part of the protocol is not marked to be filmed. To be able to assure the efficient reproduction of the method by researchers who are not familiar with hollow fiber membrane fabrication, it would be good if the authors add to the associated parts of the protocol a note (section 2 or 3), to guide the reader on if the Spinneret is operated exactly in the form it was received from the vendor, if/what modifications have been applied or if such fibers could alternatively be purchased directly from the companies.

**The authors have added language in the discussion clarifying that all components necessary for the hollow fiber membrane system are commercially available. The spinnerets themselves are available can be ordered from a AEI Inc., manufactured to desired dimensions listed in the Table of Specific Materials and language to this effect has been added in a note within section 1.2. A hyperlink to the manufacturer’s website with schematics of the spinneret itself has also been provided in the Table of Specific Materials.**

Figure 2 is not referred to in the main text of the article except for panel A (line 296).  
Figure 4A is not referred to in the main text of the article, just panel B (line 310).

**All panels are now referenced in the text**

**Editorial comments:**  
Changes to be made by the Author(s) regarding the written manuscript:

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

**The authors have proofread the manuscript for spelling and grammar.**

2. Please add an author list and then provide the full postal address of each affiliation

**The author list has been provided and postal codes have been added to each affiliation address.**

3. Please provide an email address for each author.

**Email addresses for each author have been provided.**

4. Please spell out each abbreviation the first time it is used.

**The first instance of ECM in the long abstract has been defined.**

**The first instance of SEM in the body has been defined.**

**The title of figure 3 was rephrased to eliminate need for abbreviation.**

5. Please adjust the numbering of the Protocol to follow the JoVE Instructions for Authors. For example, 1 should be followed by 1.1 and then 1.1.1 and 1.1.2 if necessary. Please refrain from using bullets, dashes, or indentations.

**Section numbering has been modified to conform to the JoVE Instructions for Authors**

6. Please revise the protocol to contain only action items that direct the reader to do something (e.g., “Do this,” “Ensure that,” etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as “could be,” “should be,” and “would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note.” Please include all safety procedures and use of hoods, etc. However, notes should be used sparingly and actions should be described in the imperative tense wherever possible.

**Several steps have been corrected to use the imperative tense. Mislabeled notes have been appropriately labeled.**

7. Please add more details to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol. 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.

**Additional detailed steps have been added throughout the protocol sections and extra clarifying details added to several preexisting steps. Additional details referring specifically to construction of the mesh scaffold displayed in figure 5 has been added under sections 1.5 and 2.1 of the protocol. An authoritative reference on hollow fiber membrane characterization has also been added.**

Some examples:

A schematic of the spinneret as Figure 1 would greatly aid in the protocol.

**A process flow diagram of the hollow fiber membrane casting system has been included as figure 1. A hyperlink to the manufacturer website with detailed schematics and descriptions of the spinneret itself have been added to the list of materials.**

4.1: Please specify the incubation temperature.

**Incubation temperature has been provided.**

4.4: How large is the petri dish?

**Diameter of the petri dish has been provided (6 cm).**

4.5: Please specify the culture conditions.

**Additional culture condition information has been provided.**

8. Figure 1: Please use the lowercase x to denote the number of times the step should be repeated.

**Figure 1 caption has been amended to use the lowercase x convention.**

9. Please also describe other panels of Figures 2 and 4 in the Representative Results.

**All figure panels are now referenced in the text.**

10. References: Please do not abbreviate journal titles.

**Journal title abbreviations have been amended except where abbreviations are used in the official title of the journal (e.g., “The AAPS Journal).**