

2018.10.24


Dear Editors,

We thank the reviewers for their generous suggestions regarding our manuscript JoVE58932-Wet Spinning of Gelatin for Tissue Regeneration and we have modified the manuscript to address their concerns. In this revision, we also want to declare that the title of the manuscript was revised into “**Wet Spinning-based Molding Process of Gelatin for Tissue Regeneration**”.

We hope that the revised manuscript may be suitable for the publication in JoVE. We are looking forward to hear from you.

Thank you in advance.

Sincerely,

A handwritten signature in black ink, appearing to read 'Sheng Tang Wu', is written over a horizontal dotted line.

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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.

Answer: Noted.

2. Please note that numbering of institutional affiliation should follow the order of authors. First author gets 1, next author with different affiliation gets 2, etc., following from first to last.

Answer: Noted.

3. Please include an ethics statement before your numbered protocol steps, indicating that the protocol follows the animal care guidelines of your institution.

Answer: Already added. Page 5 line 187-188 and page 7 line 279-281.

4. 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. See examples below:

Answer: Revised, and the numbering of protocol steps was changed due to the revision.

5. 2.1.1: A scheme showing the spinning setup would be helpful.

Answer: Already added.

6. 2.1.3: What volume of acetone solution is needed? What container is used?

Answer: Revised. Beaker glass containing 100 mL of 99.5% acetone solution.

7. 2.1.4: What is the injected solution?

Answer: Revised. Gelatin solution.

8. 2.1.5: What fibers? This step is unclear.

Answer: Revised. Gelatin fibers.

9. 3.1: Please mention how to coat the surface with gold. What is the unit for 60? How to prepare the sample for SEM?

Answer: Revised. Coating with gold for 60 seconds by using ion sputter coater

machine.

10. Lines 151-152: Please move the ethics statement before your numbered protocol steps.

Answer: Revised.

11. 4.1: Please specify the source/type of fat tissues. What container is used? What volume of the transfer solution is needed?

Answer: Revised. The source of fat tissue is oral adipose tissue, use petri disk containing a 10 mL of transfer solution.

12. 4.2: What is used to cut?

Answer: Revised. Scalpel blade.

13. 4.4: What container is used? Please specify throughout.

Answer: Revised. 15 mL plastic tube.

14. 4.5: What is the temperature? What container is used?

Answer: Revised. 15 mL plastic tube, stand for 1 day at 37°C in humidified atmosphere containing 95% air and 5% CO₂.

15. 4.6: Please provide centrifugation parameters and composition of stem cell medium.

Answer: Revised. Centrifuge the sediment at 500xg for 5 min, transfer it into T25 culture flask containing stem cell medium (consisting DMEM, 5% of FBS, N-acetyl-L-cysteine, ascorbic acid-2-phosphate, 1% antibiotic/antimycotic, and insulin).

16. 4.7, 5.2.2: What volume of trypsin-EDTA is added?

Answer: Revised. 1 mL.

17. 4.8, 5.2.3: What volume of FBS is added?

Answer: Revised. 1 mL.

18. 4.9, 5.2.4: Please convert centrifuge speeds to centrifugal force (x g) instead of revolutions per minute (rpm). Please specify the volume of stem cell medium.

Answer: Revised.

19. 5.1: Please specify which tube is used here.
Answer: Revised. Gelatin tube.
20. 5.3, 6.4, 6.11: What is the incubation temperature? Please specify throughout.
Answer: Already specified.
21. 6.11: What volume of Hoechst 33342 is added?
Answer: Revised. 1 mL.
22. 7.1.1: Please specify the age, gender and type of rat. Please mention how proper anesthetization is confirmed.
Answer: Revised.
23. 7.3.1: Please specify the surgical instruments used. How large is the incision?
Answer: Revised. 2 cm of wound.
24. 7.3.2: What material is placed?
Answer: Revised. Gelatin tube.
25. 7.5.1: What is the incubation temperature? What is the concentration of CO₂?
Answer: Revised.
26. 7.5.2: How to remove the implanted tissue? What tool is used?
Answer: Revised.
27. Please combine some of the shorter Protocol steps so that individual steps contain 2-3 actions and maximum of 4 sentences per step.
Answer: Noted.
28. After you have made all the recommended changes to your protocol (listed above), please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol.
Answer: Noted.
29. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense. Please do not highlight any steps describing anesthetization and euthanasia.

Answer: Noted.

30. Please include all relevant details that are required to perform the step in the highlighting. For example: If step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be highlighted.

Answer: Noted.

31. Figure 1: Please indicate the magnification used in panel C.

Answer: Revised the figures in this manuscript. The figure 1C become the figure 2C.

32. Figure 3: Please indicate what the blue and green colors stand for in the figure legend.

Answer: Revised.

33. Figure 4: Please explain what the red rectangle stands for.

Answer: Revised.

34. Please provide figures with higher resolution.

Answer: Noted.

35. JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations:

- a) Critical steps within the protocol
- b) Any modifications and troubleshooting of the technique
- c) Any limitations of the technique
- d) The significance with respect to existing methods
- e) Any future applications of the technique

Answer: Revised.

Reviewer #1:

1. The title is incorrect - as the authors are not 'wet spinning in gelatin...' or is 'of gelatin, not 'in'. Seems to be confusion in the script as to how fibres and tubes were spun, more specifically the link between them; was it that the fibres were made and then a tube dipped into a fibre solution or that fibres is one thing and tubes are another?

Answer: The title of this manuscript was currently revised. The fabrication steps of gelatin fibers and gelatin tube are two different things. The gelatin fibers were made by wet spinning and then by employing the basic concept of the wet spinning method, we developed the gelatin tube with another protocol (molding process), which utilize the shape of the material as desired to increase the functionality of its surface and to mimic the features of human tissues. The confusion part of the manuscript regarding to this issue was already revised.

2. The term 'pure gelatin' should be changed to 'gelatin' as there are always impurities and nearly always endotoxins contained in gelatin solutions.

Answer: Revised.

3. If tubes are the desired form then why were tubes not directly extruded? Does the rolling up and the fact that gelatin can be remodeled quickly in vivo affect tube patency? For cardiovascular applications burst strength will be low for rolled up tubes.

Answer: The idea of tube fabrication is coming from the concept of wet spinning of gelatin fibers, thus we firstly explored about the wet spinning of gelatin fibers then the gelatin tube one. As tissue regeneration application, we chose the gelatin tube form in the case of its functionality surface and to mimic the features of human tissues. However, intrinsically, we also can develop any shape of material from gelatin fibers, *e.g.* gelatin conduit that rolled-up from several gelatin fibers, for the other desired application.

4. Part of the last sentence '...and its implementation in the laboratories is expected to greatly benefit the enhancement of tissue regeneration' is conjecture and should be removed.

Answer: Revised.

5. The first paragraph of the introduction is misleading, in fact autograft for nerve for some injuries is gold standard and does function. Revise.

Answer: Revised.

6. The source of the gelatin needs defining - porcine? Bloom strength? Gelatin A or B? At least this should be put in supporting information.

Answer: Gelatin type B derived from porcine. Already added in supporting information.

7. The 5% concentration is that w/v%?. Define.

Answer: 5% weight per volume solution. Revised.

8. Gelatin is thermoreversible, dependant on concentration too - at what temperature was the gelatin spun at and indeed comment should be made about the temperature range/concentrations that are suitable for this spinning.

Answer: The fabrication of gelatin-based material in this protocol was done in the room temperature (22-26°C). Already added this condition in the discussion part.

9. Section 2.1.3. 'proper tube connector....' Makes no sense. Revise.

Answer: Revised.

10. The peristaltic pump rpm is given however better would be to declare the solution flux coming out of the needle e.g. ml/min or micro L/h whichever makes most sense.

Answer: Understood. However, the machine didn't give an information about how much rpm thus how much the solution flux coming out. So, we just declared the rpm and the type of the machine, and the flow rate depends on the rpm we used.

11. Most importantly - when gelatin is in fibre form and immersed in water (solvent for gelatin) the fibres will often reorganise and form a film. Especially above their sol-gel transition temperature. Comment should be made on this. Ergo - how stable were the fibres and is there evidence that the fibres maintained their form, else they may just have conglutinated into a tube-like structure with little fibre-like morphology.

Answer: Gelatin fibers and tube were stable in their shapes after spun or molded in the acetone solution and then we immersed it into polycaprolactone/dichloromethane (PCL/DCM) solution to maintain their shapes for the *in vitro* and *in vivo* examination. We have added these new steps in the protocol.

12. The link between fibre formation 2.1 and tube formation is unclear - it seems that

tubes are formed from gelatin solution and not fibres. Revise for clarity.

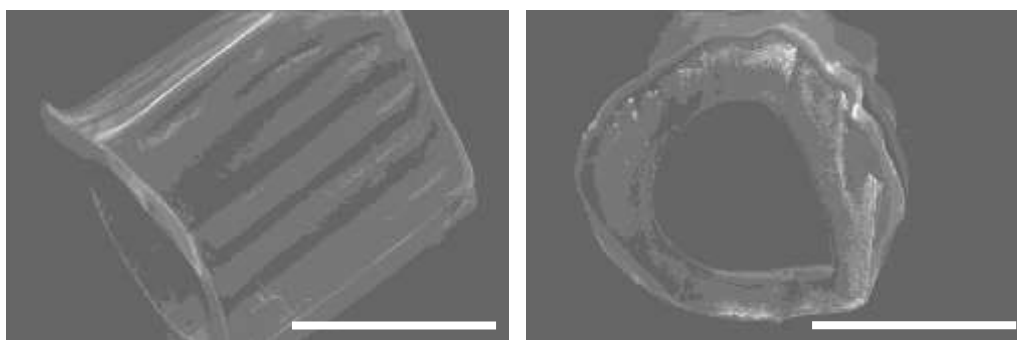
Answer: Revised. The fabrication of gelatin fibers and tube are two different things.

13. There are details missing in 3.1 - 60 what? Also dry fibers when immersed in water will likely re-swell so how relevant is the SEM? Would the tubes be dried before use?

Answer: 60 seconds, already revised. The SEM test didn't require the water immersion of gelatin materials. Right after spun or molded, the gelatin materials were dried in the room temperature and followed the SEM test protocol.

14. SEM of the tube after immersion in PBS - similar to the time points of the cell culture study should be shown, to evidence how the fibres reorganise and if the tube collapses and fibres conglomerate forming a film/solid gel mass.

Answer: The fabrication of gelatin fibers and tube are two different things. The idea of tube fabrication is coming from the concept of wet spinning of gelatin fibers, and then by employing this basic concept, we developed the gelatin tube with another protocol (molding process). Hereby we attached the SEM of the tube after immersion in PBS (scale bar: 500 μ m), but we didn't perform SEM for the fibers one.



15. The discussion section: '...including ant semi-crystalline or amorphous state of collagen' makes little sense - may be an English issue here; in any case there will indeed likely be helical structure formation. Revise.

Answer: Already revised based on reference.

16. The endotoxin issues around gelatin are not mentioned. They should be as this is one disadvantage to using gelatin.

Answer: Gelatin has been well-known and widely used in the food, pharmaceutical, and cosmetic industries due to their excellent biocompatibility

weak antigenicity, so we thought that the endotoxin issues around gelatin is the thing need to be focused to reduce, such as the usage of gelatin powder with high quality, but not the part of disadvantage.

17. The discussion section mentions work by Kulkarni et al. however there are numerous examples of biopolymers being formed into fibres using simple equipment. The statements in the discussion are inflated and inaccurate and should make mention of other biopolymers that are spun, such as cellulose (including Lyocel), cellulose nanocrystal and fibrils, - alginate (also commonly made as fibres), and there are quite a number of examples with gelatin and gelatin/alginate e.g. CY Yang 2009. <https://doi.org/10.1080/10731190903041022> also see: ADVANCED FUNCTIONAL MATERIALS 2013 Volume: 23 Issue: 3 Pages: 346-358 DOI: 10.1002/adfm.201201212

Answer: Revised.

18. Often gelatin should be cross-linked to maintain its form, yet no crosslinking has been performed here - therefore did the fibre structure reorganise to form a film/solid mass.

Answer: The polycaprolactone/dichloromethane (PCL/DCM) solution post-treatment was done so gelatin-based materials made by this protocol can maintain their shapes without any re-organization. We have added these new steps in the protocol.

Reviewer #2:**Major Concerns:**

Generally, the present research work is appropriate, innovative and could be acceptable. However, some modifications are necessary to be made which some of the most important issues are mentioned below and it would be acceptable and publishable after doing the modifications.

1. Page 2, Line 62: Title "1" should be put for introduction.

Answer: Based on the journal's template, there's no numbering order for each section.

2. Page 2, Lines 85 and 86: This sentence is not correct in general, it should be corrected and a reference(s) should be added.

Answer: Already revised and added reference.

3. Page 3, Paragraph 1, Lines 88, 89, 90 and ...: In general, solution spinning processes like wet spinning are more difficult and more expensive in fiber production industry compared to melt spinning and if there is a possibility of melt spinning for a specific polymer, industrialists always prefer melt spinning for producing fibers. Therefore, the pointed matter from the previous page to here, is necessary to be corrected in terms of contents and according to the mentioned point.

Answer: Understood and revised.

4. As it is mentioned in the article title, the present research is based on wet spinning, thus, some points in wet spinning logic and principles should be mentioned in introduction so that the readers of the article have an accurate understanding from this section. Since the respected authors are the researchers in medical and pharmaceutical field, they could use the following papers for better guidance:

1. "Exploring the effects of non-solvent concentration, jet-stretching and hot-drawing on microstructure formation of poly (acrylonitrile) fibers during wet-spinning." *Journal of Polymer Research* 18, no. 6 (2011): 1343-1351.

2. "Simultaneous effects of polymer concentration, jet-stretching, and hot-drawing on microstructural development of wet-spun poly (acrylonitrile) fibers." *Polymer Bulletin* 66, no. 9 (2011): 1267-1280.

3. "Designing index of void structure and tensile modulus in wet-spun poly (acrylonitrile) proto-fibres. Part II: synergistic effect of dope non-solvent concentration and jet draw ratio." *Iranian Polymer Journal* 17, no. 3 (2008): 227-235.

4. "The synergistic effect of dope concentration and jet drawing on structure development of wet-spun poly (acrylonitrile)." e-Polymers 8, no. 1 (2008).
5. Designing index of void structure and tensile properties in wet - spun polyacrylonitrile (PAN) fiber. I. Effect of dope polymer or nonsolvent concentration." Journal of applied polymer science 109 (6), 3461-3469 (2008).

Answer: Revised based on the suggestion.

5. Page 3, Line 104: It is better to use "Experimental" instead of "Protocol".
6. Page 3, Line 104: 2. Experimental.

Answer: The use of "Protocol" is based on the journal's template.

7. Page 3: Specifications of the used Instruments for samples characterization such as the manufacturer company, the device model, etc. for all devices like SEM are necessary to be mentioned precisely in Experimental section.

Answer: Revised.

8. Page 3:

2.1. Solution preparation, 2.2. Wet spinning, 2.2.1. Fiber formation, 2.2.2. Tube formation ...

Answer: Revised.

9. Page 3, Line 115: The schematic shape of the spinning machine with the mentioned specifications is necessary to be added to this section.

Answer: Revised by adding the scheme of wet spinning setup.

10. Page 7, Line 271: What is the purpose of showing A, B and C in Figure 1? While A, B and C are not referred in the Results section.

Answer: Revised.

11. Page 7, Figure 2: The SEM images do not have any scale in order to investigate the accuracy of the mentioned claims in Figure 2.

Answer: Revised the SEM images.

12. Page 6, Line 289: The place of Figure caption is not here, the end of the article after the references section is appropriate.

Answer: Revised.

13. Page 7, Results section: Little and limited information is available, however further discussion is possible.

Answer: Revised.

14. Page 8, Lines 310-333: This is a part of the introduction and should be transferred to introduction section.

Answer: Revised based on suggestion.

15. Page 8, Lines 335-351: Regarding to the figures and the results contents, more comprehensive discussion could be made here.

Answer: Revised.