Dear Dr. Nguyen,

Thank you very much for your letter and advice on our manuscript Preparation, Procedures and Evaluation of Platelet-Rich Plasma Injection in the Treatment of Knee Osteoarthritis. In the current form, we have modified our manuscript according to the editorial and peer review comments.

**Editorial and production comments:**

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

Advice 1: Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

**Answer 1:** **Thanks very much for the comment. As suggested, we have checked the manuscript again to ensure that there are no spelling or grammar issues.**

Advice 2: For in-text formatting, corresponding reference numbers should appear as numbered superscripts after the appropriate statement(s).

**Answer 2: Thanks very much for the comment. As suggested, we have changed the in-text formatting.**

Advice 3: JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents.  
For example: Corning, Eppendorf, etc.

**Answer 3:** **Thanks very much for the comment. As suggested,** **we have removed all commercial language from the manuscript.**

Advice 4: 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.

**Answer 4:** **Thanks very much for the comment. The changes are marked in red color throughout the revised manuscript.**

Advice 5: What are the inclusion/exclusion criteria of the patients?

**Answer 5： Thanks very much for the comment. The inclusion/exclusion criteria of the patients have been added in the revised manuscript, marked in gray color shadow.**

**Inclusion criteria: patients with knee osteoarthritis who have poor pain relief for simple analgesic medication (such as acetaminophen) and conservative treatment.**

**Exclusion criteria: patients with venous return or lymphatic drainage disorder; patients with knee joint infections; patients with** **a** **dermatosis or infection in the injection area; patients with fever; patients with coagulant function abnormality; patients with serious cardiovascular disease.**

Advice 6: Please do not abbreviate journal titles.

**Answer 6:** **Thanks very much for the comment. The changes are marked in orange color throughout the revised manuscript.**

**Changes to be made by the Author(s) regarding the video:**

Advice 1: Please increase the homogeneity between the video and the written protocol.

**Answer 1: Thanks very much for the comment. In current version, we have made the written protocol more detailed and increased the homogeneity between the video and the written protocol, marked in red color throughout the revised manuscript.**

Advice 2: Please stabilize the video images whenever possible. The camera work is very shaky throughout.

**Answer 2: Thanks very much for the comment. We feel very sorry that we did not stabilize the video images in the previous edition. We have executed anti-shake processing and re-recorded the very shaky part of the video. We think it can clearly convey the content of the protocol in current edition. However, please feel free to tell us and we will re-record the whole video if you think it is necessary.**

Advice 3: Are gloves required throughout? There is no glove usage during the preparation of the heparin syringe.

**Answer 3:** **Thanks very much for the detailed comment. We agree that glove can protect the operator. However, drawing medicine from an Ampule is a very common operation and it is normally considered that gloves are not necessary to draw medicine in China.**

**Sterile gloves are required during the preparation of the centrifugation and the injection and we have mentioned in the protocol.**

Advice 4: There are some words that are still mispronounced: centrifugation, JoVE (one-syllable), etc.

**Answer 4: Thanks very much for the detailed comment. We have invited a native English speaker from New York to record the audio. The reason why there are still some mispronunciations is he is not familiar with the medical terminology. Anyway, we have revised the mispronunciations in the current edition.**

Advice 5: 1:50 - "The" should be removed from this text overlay.

**Answer 5:** **I feel very sorry about this fault. We have corrected it in the current edition and check the video to make sure there is no other similar mistake like this.**

Advice 6: The black text against this background is a bit difficult to read. We recommend either lightening the background, or making the text white and adding a black drop shadow.

**Answer 6: As suggested, we added a white shadow to lighten the background.**

Advice 7: It appears that it is an actual patient being demonstrated upon in the video. We do see the patient's face in some of the shots. Does the patient's face need to be obscured for privacy reasons?

**Answer 7: Thanks very much for the detailed comment. Actually this patient is also an author (Hua Liu) of this article. And he is very willing to act in the video. Thanks for protecting patient’s privacy again.**

**Reviewers' comments:**

Advice 1: Please, in the video, improve the sound quality of narration voice

**Answer 1: I feel very sorry about some unclear voice. We have improved the sound quality especially in Conclusion part in the current edition.**

Advice 2: Remove commercial image of PRP kit, because a commercial kit it is not used.

**Answer 2:** **T****hanks very much for the comment. As suggested, we have removed the commercial image of PRP kit.**

Advice 3: Please improve the quality of graphics of the "Point V" - Representative results.

**Answer 3: Thanks very much for the comment. As suggested, we have improved the quality of graphics of the "Point V" - Representative results.**

**In Abstract：**

Advice 1: "osteanaphysis" is a correct term, but it is not usual. Please replace by " bone regeneration" or similar.

**Answer 1:** **Thanks very much for the comment.** **The changes are marked in yellow color shadow throughout the revised manuscript.**

Advice 2：Please clarify the meaning of "The centrifuge method in preparation included 3 types"

**Answer 2：I feel very sorry about this. Actually, what we mean is that** **there are roughly 3 types centrifugal methods to prepare PRP, including manual** **centrifugation, equipment-based centrifugation, or plasma filtration techniques.**

**The changes are marked in cyan color shadow in Introduction part.**

**In protocol：**

Advice 1: The anticoagulant used is heparin sodium. Why the authors choose this type of anticoagulant? It is not usual in PRP technology, in contrast to sodium citrate, or ACDA.

**Answer 1: Thanks very much for this important comment. Sodium citrate is also usual to be used to replace heparin sodium in my hospital, and we have added this information in the revised manuscript, marked in green color. However, heparin sodium is also efficient for impedance aggregometry in PRP preparation, according to some research and our experience 1 2. Actually we are researching about the anticoagulants selection in PRP preparation in another study.**

Advice 2: In 1.5.4. please change "karyocyte" to leukocyte.

**Answer 2: We agree with the reviewer and the change are marked in blue colour.**

Advice 3: The PRP is activated with thrombin and/or CaCl2?

**Answer 3: The PRP is not activated with any activating-agents in this protocol. According to some articles, some PRP is harvested and directly injected into the area of injury.** **In general,** **PRP used to relieve chronic inflammation or “wear and tear” injuries is usually injected without an activating agent3. Thanks very much for the comment. The changes are marked in red color shadow in Discussion part.**

**In Reference:**

Advice: Please unify the references style. For example, name and surname in references #3 and #5 and no in #1 and #2

**Answer: Thanks very much for the comment. As suggested, we have unified the references style.**

**In Figures:**

Advice: Figure 1: Please remove the commercial kit for PRP collection (the same in video), because the authors use a noncommercial system for obtaining PRP.

**Answer: Thanks very much for the comment. As suggested, we have removed the commercial kit for PRP collection in Figures and in video.**

We hope that the revision is acceptable and look forward to hearing from you soon.

With best wishes,

Ziming Chen

**Reference：**

1 Solomon, C. *et al.* Influence of the sample anticoagulant on the measurements of impedance aggregometry in cardiac surgery. *Medical Devices (Auckland, N.Z.).* **1** 23-30 (2008).

2 Zhou, S. F. *et al.* Autologous platelet-rich plasma reduces transfusions during ascending aortic arch repair: a prospective, randomized, controlled trial. *The Annals of Thoracic Surgery.* **99** (4), 1282-1290, doi:10.1016/j.athoracsur.2014.11.007, (2015).

3 Cohn, C. S. & Lockhart, E. Autologous platelet-rich plasma: evidence for clinical use. *Current Opinion in Hematology.* **22** (6), 527-532, doi:10.1097/MOH.0000000000000183, (2015).