Dear editor,

Thank you very much for your reviewing our manuscript and giving us so much good suggestions. We have carefully revised our manuscript and corrected them point by point.

Thank you again!

**Editorial comments:**  
Changes to be made by the Author(s) regarding the written manuscript:  
1. Please edit the manuscript to be clear and free from grammatic mistakes.

**Thank you. Done as suggested.**

2. Figure 1: Please line up the panels better. Some panels are off-set in Figure. Please ensure that the panels are of the same dimensions if possible.

**Done as suggested.**

3. Please delete F1/F2/F3 etc. in the panel labels, i.e., use only a, b, c, etc. to label the panel.

**Done as suggested.**

4. Please revise the title to be more concise if possible.

**Thanks. Done as suggested.**

5. Please provide an email address for each author.

**Thanks. Done as suggested.**

6. Please rephrase the Short Abstract to clearly describe the protocol and its applications in complete sentences between 10-50 words: “Here, we present a protocol to …”

**Thanks. Done as suggested.**

7. Please revise the Long Abstract to focus on the method being presented rather than the results of a specific experiment. A detailed overview of the method and a summary of its advantages, limitations, and applications is appropriate. Please focus on the general types of results acquired.

**Thanks. Done as suggested.**

8. Please expand your Introduction to include the following: The advantages over alternative techniques with applicable references to previous studies; Description of the context of the technique in the wider body of literature; Information that can help readers to determine if the method is appropriate for their application.

**Done as suggested.**

9. Please use SI abbreviations for all units: L, mL, µL, h, min, s, etc.

**Thanks. Done as suggested.**

10. Please include a space between all numbers and their corresponding units: 15 mL, 37 °C, 60 s; etc.

**Thanks. Done as suggested.**

11. 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: Lianhelihua, Acuson TM S2000, Siemens Medical Solutions, JEM-1400Plus, JEOL Ltd., etc.

**Done as suggested.**

12. 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.

**Done as suggested.**

13. 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.

Thank you. **Done as suggested.**

1.3: Please describe how ether anesthesia is done and how proper anesthetization is confirmed. How many rats are included in the experiment? What concentration of the PA/NPSi suspension is used in this step? How to ensure that the suspension is instilled to the lung of the rats?

Have given more information.  
2.2: Please describe how to perform and maintain anesthesia. Is shaving gel used before removing the rat’s hair? Which area of rat’s hair is removed?

Have given more information.  
4.1: Please describe how to open and inspect the rat’s bilateral pleural cavities. Please specify all surgical instruments used.

Have given more information.  
  
14. Please reference Figure 1, Video 1, and Video 2 in the Representative Results section.

**Done as suggested.**

15. As we are a methods journal, 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

These are very good idea, we have revised the manuscript.   
16. References: Please do not abbreviate journal titles.

**Done as suggested.**  
17. Please revise the table of the essential supplies, reagents, and equipment. The table should include the name, company, and catalog number of all relevant materials in separate columns in an xls/xlsx file.

**Done as suggested.**

**Reviewers' comments:**  
  
**Reviewer #1:**  
  
Manuscript Summary:  
The underestimation of nanotoxicity may induce that some diseases are considered as "idiopathic". In fact some times nanoexposure may induce granulomatosis or inflammatory diseases . It's important to have animal models to test nanosafety and study and look for nanoparticles in the biopises of the tissue or on effusions such as pleural or pericardial fluids.  
  
So the model in rats with polyacrylate/nanosilica and the technics for isolating nanoparticles in the pleural effusion is very intersting for other searchers needing reproducing it.  
  
Major Concerns:  
Ther is no major concern  
  
Minor Concerns:  
It would be good to have repeated explainments about the synergy between polyacrylate and nanosilica. Why not test only nanosilica toxicity? Is there a synergistic effect between nanosilica and polyacrylate..???We need more détails about that.  
Thanks. We will try and do further tests just by giving rats nanosilica  
  
**Reviewer #2:**  
  
Manuscript Summary:  
The authors describe generation of pleural effusion in a rat model with subsequent detection of polyacrylate/nanosilica nanoparticles in the effusion.  
  
Major Concerns:  
There are some open questions regarding the application of the model. Is this method intended as a toxicity test model for nanoparticles and chemicals? Relevance of the model for the human situation is questionable as only very high doses, >2g polyacrylate/nanosilica particles in the lung of the standard reference human, cause the effect. How reproducible/variable is the system.

Our test shows that the model is very effect and it can be used for nanotoxic study or pulmonary diseases with pleural effusion

Minor Concerns:  
Description of the protocol (phrasing) is unusual.  
We have revised the protocol.

**Reviewer #3:**  
  
Manuscript Summary:  
This study by Wen Cao and colleagues exposed the method to make pleural effusion by intratracheal instillation of polyacrylate/nanosilica (PA/NPSi) and how to isolate these nanoparticles in pleural effusion. This study is helpful to understand the potential toxicity of nanoparticles. The method used is clearly exposed and describe. However, some results have to be precise.  
  
Major Concerns:  
  
1) According to the title, this study is about pleural effusion induced by PA/NPSi exposure. However, the authors conclude also in pericardial effusion which is not described as well as the pleural effusion in this study. In particular, there are no descriptions of pericardial effusion on CT scanner and no confirmation by dissection of rats. This data should not appear in the conclusion of the abstract.

Thanks. we reviewed and modified the abstract

2) I understand that the group with 3.125mg/kg of PA/NPSi is used as a control group. It should be clearly describe in the methods.

Thanks, We have revised the protocol.  
3) At day 14 the authors described signs of adhesion of the pleura in all groups with ultrasound examination which is not confirmed in the section "dissection/inspection" of pleural effusion. Please clarify.

Ultrasound examination is more sensitive to detect pleural effusion or pericardial effusion than just inspection

4) Figure 1, it could be helpful if there was some annotations on the figure to understand the different anatomical structures on the images exposed.

Thanks. The videos with arrow should in the manuscript give a hand to understand

5) Figure 2a,b is unclear. A pleural effusion is normally described as a declivitous, elliptic or crescent-shaped which is not found on this figure. In addition there is no annotation or arrows on the figure which may help readers who are not used to this technique.

Figure 2a,b are representative images of thoracic CT images in rats. Signs of a pleural effusion normally described as a declivitous, elliptic or crescent-shaped were not observed is that becuase CT scanning is not sensitive as Ultrasound examination. The amount of pleural effusion in rats is very small.

6) Concerning the overall presentation of the results it might be useful to add synthetic(s) additional(s) figure(s) to summarize your results obtain with the different groups of rats and the different techniques.

Thanks.

7) In the discussion, do you have any hypothesis concerning the spontaneous resolution of pleural effusion after 14 days? What do we know about the elimination of PA/NPSi in this model?

After 14 days, pleural effusion was observed and we can’t find anymore.

8) Line 145, in this protocol there was no histological findings of granuloma or fibrosis, this result is different from your clinical study on patients. This is probably related that the duration, concentration and the type of exposure were different between the two models. It is therefore difficult to compare the two models without giving the limits.  
Thanks. It is very good suggestion.

9）as for others, we have carefully revised and corrected them point by point.