

POINT BY POINT RESPONSE

1. Is it ok to place this in Immunology and infection classification rather than in Medicine?

Answer: Yes. It's ok to place this in Immunology and infection classification.

2. The results shown are with *P. fluorescens*, please change this here and throughout.

Answer: P. fluorescens Migula was used in flowcytometry and fluorescent microscopic experiments. P. aeruginosa (strain PAO1) was used in all other experiments throughout the manuscript.

3. *P. aeruginosa* can be detected in alveolar sacs as early as 1 h after the infection in animal models. Citations?

Answer: Citation has been added.

4. (It has been reported that lung epithelial cells may regulate microbial invasion, colonization, or replication independent of immune cells.) Citations?

Answer: Citation has been added.

5. Since figure 1 schematics show robot smoking please discuss this part in the introduction, please include the significance of using CSE method instead. Please include citations for the claims.

Answer: We have discussed this robot smoking method in Introduction.

6. Please ensure that each section has a representative result.

Answer: We have presented representative result of each important section.

7. How is this done?

Answer: We suck the air that containing cigarette smoke from 10ml to 50ml scale to get a 40ml volume of smoke.

8. (*P. fluorescens* Migula (strain PAO143).) Somewhere in the introduction please also include the significance of using this strain for some of the sections to bring out clarity. Please also include citations.

Answer: This strain P. fluorescens Migula (strain PAO143) is used in flow cytometry experiments. P. aeruginosa PAO1 was used in all other experiments. We have added a sentence and included a citation in Introduction Paragraph 2 Line 76.

7. Do you use single colonies in this case?

Answer: We don't use the single colonies. We collected pseudomonas in the plate with a policeman to get more bacteria for culturing in tube.

8. Where was this cell line used? If presenting as a primary cell culture model, please include representative results for this as well. If not, please discuss in the discussion as a limitation and how this could be helpful.

Answer: The primary human airway epithelial cell has been used to treated with cigarette smoke. We have added the data of HSAEC in the representative results as new Figure 3.

9. Please include some results to show that cells are indeed viable after the treatment with CSE.

Answer: Results of cell viability under CSE treatment have been presented as new Figure 4.

10. Please include results for this section... what happens to the cells after 1 h of bacteria/CSE treatment? Do they die?

Answer: We have included viability results in the representative results as new Figure 5. Essentially, we did not observe substantial cell death in this experiment.

11. (NOTE: To confirm the internalized bacteria, cells infected with *P. fluorescens* Migula were observed under confocal microscopy.) Please include a Result figure. Also, this can be moved to the representative result section.

Answer: A representative result of P. fluorescens Migula under fluorescent microscopy has been added as new Figure 9.

12. What is used as a control? How do you ensure that the increase is not because of the lung cell number?

Answer: We used equivalent amount of total RNA in reverse transcription and equivalent amount of cDNA for quantitative PCR to avoid potential errors from experimental design.

13. Results can be reprinted from previous publications if reprint permission is obtained and the figure is cited appropriately in the Figure Legend, i.e. "This figure has been modified from [citation]."

Answer: Thanks for your kind suggestions. We didn't use previous published data.

14. Details on how to perform Robot smoking procedure is not present in the protocol and no results are presented.

Answer: We have added the Robot smoking exposure procedure in protocol in protocol 3.9-3.11. We introduce this method in the protocol but without representative result.

15. Which strain?

Answer: It is P. fluorescens Migula (strain PAO143).

16. Citation for this claim?

Answer: Citations have been added at Line 351-353.

17. Please discuss why this should be done? Why is this a better approach? Please use citations.

Answer: Primary lung epithelial cell monolayer culture and robot smoking in air-liquid interface mimic the physiological cigarette smoke exposure and it's a cellular model other than conventional cell culture to understand cellular responses to cigarette smoke challenge. Besides, citation has been added.

18. Citations.

Answer: Citation has been added.

19. Please bring out clarity on this.

Answer: We have rewritten this sentence as “This approach may mimic human infection in a physiological way and through this approach we may gain insights in lower respiratory bacterial infection”.