

Dear Reviewers,

Thank you very much for your valuable comments and feedback regarding our research paper. Each of your insights have served to strengthen our manuscript and we have made changes to reflect them. We have made the changes directly to the manuscript, as well as recorded the changes below.

Comments from Reviewers:

Reviewer #1:

- I was impressed by this interesting and excellent work. Using simulation models to demonstrate situations similar to those experienced by living subjects results in a correctly scientific approach, studying presumed physiological or pathological conditions in simulator models prior to their application in humans. In this work, the artificial lung conditions of patients with or without intubation are carefully reproduced, being able to demonstrate experimentally that IPV improves the mucus clearance in simulated models.

Reviewer #2:

- This is an interesting and well-conducted bench study. I have only one comment. Under-humidification was recognized as a potential problem when applying IPV during mechanical ventilation (MV) (Ref). Suboptimal humidification caused by the high inspiratory flow rate and gas decompression during IPV may increase the risk of airway obstruction by secretions. Dellamonica et al. have shown that adequate absolute humidity could only be provided by placing the heated humidifier on the inspiratory line downstream of the IPV device. The heated humidifier seems not included in the experimental circuit utilized in this study. Was under-humidification a likely explanation for the distal displacement of mucus into the lungs on the IPV-MV model? Ref: Dellamonica et al. Intrapulmonary percussive ventilation superimposed on conventional ventilation: bench study of humidity and ventilator behaviour. Intensive Care Med. 2008;34(11):2035-43.

Answers/Responses to Reviewers:

Reviewer #1:

- Thank you for your kind words.

Reviewer #2:

- Thank you for your valuable feedback. We have made your recommended changes. The excerpt below was included in the manuscript within the discussion section:
A third potential limitation in our study was that although we controlled for humidification by conducting our experiments in a tightly controlled environment within a room at standard humidity, we did not attach a humidifier to our experimental circuit separately. Dellamonica et al (2008) found that the optimal way to effectively humidify this circuit was to attach a humidifier downstream from the IPV machine.^[40] Dellamonica et al (2008) recognized that when IPV is combined with invasive mechanical ventilation, the production of high

inspiratory flow rates and gas decompression prevented optimal humidification and warming of the inspired gas.^[40] This combination often results in the drying of mucus and the risk for airway obstruction. The question arises whether this may have caused the lack of proximal movement of the mucus in our MV model. Although this is plausible, if this was indeed the reason for the negative impact of IPV in our MV model, we would have expected the majority of the mucus to remain in the middle of the circuit where it was initially instilled, and not be displaced distally (>80% of the mucus in fact moved distally). Furthermore, because each experiment was conducted for a very short period of time (~10-minutes) the potential desiccating properties of the IPV machine should not likely have made a large impact. But regardless, further studies are needed to confirm or refute this hypothesis.

Sincerely,

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