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Low-Frequency Intrapulmonary Percussive Ventilation Increases Aerosol Penetration in a 2-Compartment Physical Model of Fibrotic Lung Disease

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1 **Low-frequency Intrapulmonary Percussive Ventilation Increases**
2 **Aerosol Penetration in a 2-Compartment Physical Model of Fibrotic**
3 **Lung Disease**

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18

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21

22 **Abstract**

23 In patients with fibrotic pulmonary disease such as idiopathic pulmonary fibrosis (IPF), inhaled
24 aerosols deposit mostly in the less affected region of the lungs, resulting in suboptimal
25 pharmacokinetics of airway-delivered treatments. Refinement of aerosol delivery technique requires
26 new models to simulate the major alterations of lung physiology associated with IPF, i.e.
27 heterogeneously reduced lung compliance and increased airway caliber. A novel physical model of
28 the respiratory system was constructed to simulate aerosol drug delivery in spontaneously breathing
29 (negative pressure ventilation) IPF patients. The model comprises upper (Alberta ideal throat) and
30 lower airway (plastic tubing) models and branches into two compartments (Michigan lung models)
31 which differ in compliance and caliber of conducting airway. The model was able to reproduce the
32 heterogeneous, compliance-dependent reduction in ventilation and aerosol penetration (using NaF
33 as a model aerosol) seen in fibrotic lung regions in IPF. Of note, intrapulmonary percussive
34 ventilation induced a 2 to 3-fold increase in aerosol penetration in the low-compliance/high airway
35 caliber compartment of the model, demonstrating the responsiveness of the model to therapeutic
36 intervention.

37 **Words in abstract: 172**

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41 **Introduction**

42 Fibrotic interstitial lung diseases (ILD) are a group of severe chronic non-communicable lung diseases
43 where excessive deposition of abnormal extracellular matrix, in association with epithelial and/or
44 endothelial lesions, results in progressive respiratory failure. Idiopathic pulmonary fibrosis (IPF) is the
45 most common and the most severe idiopathic ILD (King, Pardo, and Selman 2011). In IPF, fibrotic
46 lesions follow the heterogeneous “usual interstitial pneumonia” pattern where fibrotic lesions
47 alternate with preserved regions, and predominate in peripheral (subpleural) areas.

48 Current treatments for IPF are unsatisfactory. Although two therapies, pirfenidone and nintedanib,
49 both slow the decline of lung function (Noble et al. 2011; King et al. 2014; Richeldi et al. 2014) and
50 may increase survival (Canestaro et al. 2016), neither drug blocks or reverses the progress of disease
51 and their tolerance is fair at best (Fletcher et al. 2016). There is a need for new drugs and treatment
52 delivery routes for IPF and other ILDs.

53 IPF is spatially restricted to the lung, making it an ideal candidate disease for the use of topical, i.e.
54 inhaled drugs. Aerosolized delivery through the airways has key advantages over the oral or injected
55 routes for the administration of drugs to the lungs. Aerosolization is non-invasive and provides high
56 therapeutic index. Aerosolized drug delivery is used routinely in patients with airway diseases. By
57 contrast, the inhaled route is not currently used for the treatment of IPF, owing in part to low aerosol
58 deposition in the lung.

59 IPF is associated with multiple alterations in lung structure with potential to hinder penetration of
60 inhaled aerosols (Plantier et al. 2018). Fibrosis leads to major reductions in lung compliance (Zielonka
61 et al. 2010) which are presumed heterogeneous given the spatial heterogeneity of lesions, and which
62 result in reduced ventilation of the affected regions. Lung compliance is defined by the ratio of
63 change in lung volume (V) over change in the transpulmonary pressure (P_{tp}). Besides fibrosis of
64 alveolar regions, bronchial lesions may be observed whose nature is debated. Although the total
65 number of airways is reduced in end-stage IPF (Verleden et al. 2020), the number of visible distal
66 airways is increased in lungs with severe IPF (Verleden et al. 2020), while conducting airway volume
67 is increased by 32% in patients with moderate IPF in comparison with control subjects (Plantier et al.
68 2016) and expiratory airflow is increased (Plantier et al. 2018).

69 Anatomical and physiological alterations of the respiratory system in IPF may result in altered
70 deposition of inhaled aerosols. Similar total lung deposition, as measured by concentrations in
71 bronchoalveolar lavage fluid (Khoo et al. 2020) or planar scintigraphy (Samuel and Smaldone 2019),
72 can be achieved in IPF patients as compared to healthy subjects. However, planar scintigraphy
73 images suggest that deposition of micrometric aerosols is not homogenous in some IPF patients, in

74 contrast with healthy subjects (Kanazawa et al. 1993; Khoo et al. 2020). Similarly, deposition of the
75 nanometric aerosol Technegas was patchy and predominated in the upper regions of the lungs,
76 which are typically the less damaged areas in 2 IPF patients (Watanabe et al. 1995). We hypothesize
77 that reduced lung compliance results in reduced penetration and subsequent heterogeneous
78 deposition of inhaled aerosols in IPF.

79 At present, inhalation devices and protocols allow high aerosol penetration and deposition in
80 ventilated regions, but poor penetration and deposition in the regions of the respiratory system
81 receiving little or no ventilation. The preferential deposition of aerosols in the less damaged areas
82 needs to be overcome for satisfactory spatial targeting of inhaled therapeutics in IPF. It is therefore
83 important to develop optimized aerosolization protocols allowing to target the low compliance
84 alveolar regions in IPF. Models able to simulate IPF lungs under spontaneous ventilation are required
85 to test the efficacy of such protocols.

86 Acoustic/pulse pressure waves hold potential to increase aerosol delivery in low compliance lungs.
87 Indeed, acoustic waves increase aerosol penetration and deposition in the maxillary sinuses (Durand
88 et al. 2012; Möller et al. 2014; Leclerc et al. 2015; Moghadam et al. 2018). Maxillary sinuses are bone
89 structures, which have near-null compliance and thus receive no ventilation during the respiratory
90 cycle. Multiple experiments established that acoustic waves were most effective in the 100-300Hz
91 frequency range, close to the resonance frequency of the maxillary sinuses (Durand et al. 2012;
92 Möller et al. 2014; Leclerc et al. 2015; Moghadam et al. 2018).

93 In light of these elements, we hypothesized 1) that poor penetration of inhaled aerosols in low
94 compliance lung regions could be reproduced in a two-compartment mechanical model of fibrotic
95 lungs under negative-pressure (spontaneous) ventilation and 2) that acoustic pressure waves near
96 the resonance frequency of the model would increase aerosol penetration in the low compliance
97 compartment.

98

99 **Materials and Methods**

100 *Construction of a two-compartment model of the respiratory system*

101 A two-compartment model of the entire respiratory system (schematic in Figure 1) was constructed.
102 This model comprised a physical model of the upper airways (Alberta Idealised throat, Copley
103 Scientific, UK), a plastic tube modeling the subglottic airways down to the 4th bronchial division, and a
104 Y-piece connecting each lung compartment. Each lung compartment included a plastic tube modeling
105 the distal airways, and a mechanical lung model (Michigan Lung Model, Michigan Instruments, USA)

106 modeling the alveolar regions. Each mechanical lung model comprised 1) a first bellows connected to
107 the airway model described above (“active bellows”) and 2) a second bellows (“passive bellows”),
108 which was mechanically coupled to the first bellows, and was ventilated using a clinical ventilator
109 (Servo 300, Siemens, Germany). Thus, the first bellows was ventilated under negative pressure,
110 allowing to simulate physiological spontaneous breathing. Airway diameter and bellows compliance
111 could be individually adjusted. To simulate normal lung, in the “Control compartment” airway
112 diameter was 17 mm, and lung compliance was 100 ml/cm H₂O. To simulate fibrotic lung, in the
113 “Experimental compartment” airway diameter was 22 mm, and lung compliance was ≤ 100 ml/cm
114 H₂O. Overall, the volumes corresponded to those observed in an adult man. Filters (Anest-Guard,
115 Teleflex, France) were inserted in each compartment, at the junction of the tube modeling the
116 airways and the bellows modeling the alveolar regions.

117

118 *Distribution of ventilation in the model*

119 For characterization of ventilation distribution, the model was actuated under the following
120 ventilator settings: Frequency=10/min, inspiratory pressure between 5 and 18 cm H₂O, inspiratory
121 time = 2 seconds. These settings simulate spontaneous breathing in an adult subject. Ventilation of
122 each compartment was measured with spirometers (300 L/min, AD Instruments, Dunedin, New
123 Zealand) inserted in place of the filters (Figure 1). In addition, the resonance frequency of the model
124 were measured using a clinical forced oscillations device (Tremoflo, EMKA, France).

125

126 *NaF aerosol delivery and quantitation of aerosol deposition*

127 The model was connected to a jet nebulizer aerosol generator (Atomisor AOHBOX/ NL9M, DTF
128 medical, France) at the upper airway opening. This generator delivers an aerosol with 4-5 μm mass
129 median aerodynamic diameter. Inspiratory and expiratory valves were used. For all aerosol delivery
130 experiments, the pulmonary model was actuated with the following ventilator settings to simulate
131 tidal ventilation: Frequency = 10/min, inspiratory time = 2 sec, inspiratory pressure = 5 cm H₂O.
132 Three ml of a 2.5% sodium fluoride (NaF) solution were nebulized over 10 to 15 min. After each
133 nebulization session, the filters were dismantled. The filters were flushed with 30 ml of 2% vol./vol.
134 aqueous total ionic strength adjustment buffer (TISAB) solution (Sigma-Aldrich, Saint Louis, USA) and
135 incubated for 3 hours at room temperature. NaF was then assayed by electrochemical analysis
136 (SevenGo Pro, Mettler Toledo, USA). The quantity of NaF aerosol deposited in each filter (Control or
137 Experimental) was expressed as a fraction of the initial NaF dose that was loaded in the nebulizer.

138 *Aerosol distribution in the model*

139 Aerosol distribution was controlled for each nebulization experiments by dosing all parts of the
140 model. Aerosol deposition was quantitated at the level of the nebulizer chamber, expiratory filter,
141 construct, upper airway (Alberta throat model, trachea tube and Y-piece), and each lung
142 compartment (tubing and filter). Each section was washed with 30-250 ml TISAB and NaF was
143 assayed as described above.

144

145 *Delivery of acoustic/pulse pressure waves*

146 To generate acoustic pressure waves in the 20-500 Hz frequency range, sinusoidal signals were
147 generated with VB Generator freeware (<https://www.vb-audio.com/>, n.d.), amplified (t.amp
148 PM40C, Thomann, Germany), and transmitted to a vibration exciter (S-50009, Tira GmbH, Germany).
149 Amplifier gain was set so that acoustic pressure was identical across the frequency range, using a
150 sonometer; 80 dB acoustic waves could be generated at all frequencies. To generate pulse pressure
151 waves in the 1-10 Hz frequency range, a Pegaso (Dima Italia, Italy) intrapulmonary percussion
152 ventilation device was used. IPV devices deliver small volumes of air; the shape of the signal is not
153 sinusoidal (Toussaint et al. 2012). IPV pulse pressure was set at 40 cmH₂O and the
154 inspiratory/expiratory ration was set to 1. In a first set of experiments, the vibration exciter and the
155 IPV device were connected to the nebulizer/valves construct as shown in Figure 2A. In a second set of
156 experiments, the IPV device was connected as shown In Figure 2B following optimization
157 experiments. Both high-frequency acoustic waves and low-frequency IPV pulses were delivered to
158 the model for the duration of nebulization.

159

160 *Statistical analysis*

161 All data were expressed as means and standard deviations. Group by group comparisons were
162 performed with Student's t-test (2 groups) or ANOVA (more than 2 groups). Paired or unpaired
163 analyses were done as appropriate. Relationships between variables were assessed by linear
164 regression. Prism v5.0 (Graphpad, San Diego, USA) was used for all analyses.

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169

170 **Results**

171 *Ventilation and aerosol deposition in the model*

172 Firstly, distribution of ventilation (tidal volume) between the two compartments of the models was
173 assessed. As expected, tidal volume in both compartments of the model was a linear function of the
174 pressure setting on the ventilator, which was applied to the passive bellows of the lung model, and of
175 the compliance setting of the lung model (supplementary figure S1). For a given level of ventilation
176 pressure and compliance, tidal volume was not significantly different between compartments.

177 Then, a NaF aerosol was delivered into the model while ventilated, and deposition onto the
178 compartment filters, which assesses aerosol penetration into the respective compartment, was
179 measured. When the compliance of both compartments was set at 100 ml/cmH₂O, aerosol
180 deposition in the Experimental compartment (then characterized only by 33% larger airway) trended
181 to be slightly reduced in comparison with the Control compartment of the model (3.9+/-0.5% of the
182 loaded dose versus 3.0+/-0.3%, p=0.06 by paired analysis – Figure 3A).

183 Then, aerosolization experiments were repeated when compliance of the Experimental compartment
184 was decreased to 70, 50 and 30 ml/cmH₂O. As shown in figure 3A, decreasing compliance of the
185 Experimental compartment had two effects. First, an increase in aerosol penetration was observed in
186 the Control compartment; this increase correlated negatively with compliance in the Experimental
187 compartment ($R^2=0.48$, $p=0.012$). Second, aerosol penetration decreased in the Experimental
188 condition; this decrease followed a strong linear relationship with compliance ($R^2=0.96$, $p<0.0001$).
189 Thus, these experiments demonstrated that decreasing lung compliance in one compartment of the
190 system shifted penetration of an inhaled aerosol away from the low compliance compartment and
191 into the compliant compartment. As shown in figure 1B, aerosol penetration in the Experimental
192 compartment was strongly ($R^2=0.95$, $p<0.0001$) associated with tidal volume, supporting the
193 hypothesis that regional reduction in lung compliance and subsequent reduction in local ventilation
194 are key drivers in reducing aerosol deposition in fibrotic lungs.

195 For further experiments, the compliance of the Experimental compartment was set at 50 ml/cmH₂O,
196 so that the model could detect both increases and decreases in aerosol penetration following
197 delivery of acoustic/pulse pressure waves. Oscillometry parameters were determined under this
198 setting. Under these conditions, the resonance frequency of the system was 5Hz.

199

200 *20-500 Hz acoustic waves failed to increase aerosol penetration in the low compliance compartment*

201 Audible acoustic waves are known to increase aerosol penetration in the maxillary sinuses (Leclerc et
202 al. 2015). Thus, in a first set of experiments, 80 dB/20-500 Hz acoustic waves were delivered to the
203 nebulizer construct for the duration of nebulization using the construct shown in Figure 2A. As shown
204 in Figure 4A, audible acoustic waves had no effect on aerosol penetration either in the Control or in
205 the Experimental compartment, and the Experimental/control deposition ratio was unchanged.

206 In maxillary sinuses, matching acoustic wave frequency with the resonance frequency of the target
207 structures results in increased aerosol deposition (Durand et al. 2012). Because the measured
208 resonance frequency of the model (5 Hz) was much lower than 20 Hz, we raised the hypothesis that
209 low-frequency pressure waves were required to increase aerosol deposition. To deliver such pressure
210 waves, an intra pulmonary percussion ventilation (IPV) device was used. IPV devices offer important
211 advantages in the context of aerosol deposition in ILD as 1) they are already certified for clinical use
212 in humans including in association with inhaled aerosols, and 2) they have been shown to increase
213 aerosol penetration in a simple one-compartment model independent of compliance (Karashima et
214 al. 2019).

215

216 *Low-frequency IPV pressure waves increase aerosol penetration 2-fold in the low compliance*
217 *compartment*

218 Initial experiments using IPV (3Hz, 40 cm H₂O) delivered through the same nebulizer construct as the
219 one used for audible acoustic wave delivery showed, in comparison with absence of pulse pressure
220 waves, 1) that aerosol deposition in both the Control and Experimental compartments was
221 decreased but 2) that the ratio of aerosol deposited in the Experimental / Control compartments
222 increased from 0.16 to 0.41 (Supplementary Figure 2). Additional experiments were then conducted
223 to evaluate the effect of IPV (3 Hz) delivered through different nebulizer constructs (supplementary
224 Figure 3). As shown in Supplementary Figure 4A, aerosol deposition in the Experimental
225 compartment was increased by IPV (3 Hz) in 2 out of 6 constructs. To verify that the increase in
226 aerosol penetration was IPV-dependent and not only construct-dependent, aerosol penetration was
227 measured in constructs 3 and 4 both with and without IPV (3 Hz). IPV increased aerosol penetration
228 in the Experimental compartment in both constructs (supplementary Figure 4B).

229 Construct 3 was retained for further experiments where the effect of IPV frequency on aerosol
230 penetration was tested. As shown in Figure 4B, low-frequency IPV pressure waves in the 1-3 Hz

231 frequency range increased aerosol penetration in the Experimental compartment. The increase was
232 maximal (3-fold) for 1 Hz IPV. By contrast, 10 Hz IPV decreased aerosol penetration in the
233 Experimental compartment. IPV did not significantly increase aerosol penetration in the Control
234 compartment of the model although a trend was present (+29%, $p=0.08$).

235 Because low-frequency, high pressure IPV may be uncomfortable in man, an additional experiment
236 was conducted using low-pressure (10 cmH₂O) IPV. As shown in Figure 5, 1 Hz, 10 cmH₂O IPV
237 increased aerosol penetration in the Experimental compartment by 121% in comparison with no IPV.

238 *Aerosol distribution in the model*

239 In the normal lung model, 97.8 \pm 12.1% of aerosol was recovered (Table 1). 12 \pm 1.7% of aerosol
240 deposited in the respiratory system (including upper airways and lung models) while 85.8 \pm 10.6%
241 were recovered outside the model (including nebulizer, expiratory filter and construct). When
242 compliance was reduced in the experimental compartment to simulate IPF, 90.7 \pm 7.8% was
243 recovered owing to reduced deposition in both lung and delivery apparatus. Under 1Hz-40 cmH₂O
244 IPV conditions, 95.5 \pm 17.2% of total aerosol was recovered. In addition to a 3-fold increase of
245 aerosol deposition in the experimental compartment's filter, IPV induced increases in the construct
246 and the throat model, as well as a \approx 50% decrease in the expiratory filter.

247

248 **Discussion**

249 In this study, we developed a novel 2-compartment model of the respiratory system simulating
250 fibrotic lung disease, and we showed that heterogeneous reductions in lung compliance resulted in
251 1) an increase of aerosol penetration in the preserved lung regions and 2) proportionate decreases in
252 tidal volume and aerosol penetration in the less-compliant region. The strength of the associations
253 between lung compliance, tidal volume and aerosol penetration support the hypothesis that reduced
254 lung compliance is the main determinant of reduced aerosol deposition in fibrotic lung regions. Then,
255 we used this model to explore whether acoustic/pulse pressure waves modulated aerosol
256 penetration, and showed that low-frequency intrapulmonary pressure ventilation pulses reliably
257 increased aerosol penetration in the low compliance compartment.

258 Although the total amount of aerosol-delivered drugs may be similar in patients with fibrotic lung
259 disease and healthy controls at the level of the whole organ (Diaz et al. 2012; Khoo et al. 2020),
260 imaging studies suggest that inhaled aerosols may mainly deposit into the less-affected regions of
261 the lungs and thus presumably miss their cellular targets (Kanazawa et al. 1993; Watanabe et al.
262 1995). Despite recent demonstration that small aerosol particles (1.5 μ m diameter) and polydisperse

263 aerosols should be preferred for IPF patients (“The Topical Study of Inhaled Drug (Salbutamol)
264 Delivery in Idiopathic Pulmonary Fibrosis. - PubMed - NCBI” n.d.), preferential deposition of aerosols
265 in the less damaged regions needs to be overcome. Optimizing spatial targeting is a major challenge
266 to develop inhaled therapies for diseases which, like IPF, are characterized by heterogeneously
267 distributed fibrotic lung lesions.

268 Experimental models are required to understand the mechanisms driving aerosol penetration and
269 deposition in fibrotic lung disease. To this day, few studies were conducted in the aim of simulating
270 both disease mechanics and aerosol deposition in fibrotic lungs under spontaneous ventilation.
271 Montigaud et al. developed an *ex vivo* model combining heat-treated porcine lungs and a 3D-printed
272 replica of the human airways (Montigaud et al. 2019). Although this model better simulates a
273 branched airway anatomy, allows to replicate reductions in lung compliance predominating in
274 subpleural regions, and can be used for aerosol deposition studies, it is unwieldy and cannot be
275 reused for multiple experiments. To our knowledge, the mechanical model described herein is the
276 first to offer the possibility to simulate the geometrical and physical characteristics of either mild or
277 severe, restrictive or obstructive (using airflow resistances) lung disease *in vitro*, and to conduct
278 replicated experiments of inhaled drug delivery. Moreover, the nebulized aerosol deposited in the
279 whole of lower airways of the Normal lung model (10% of the nominal dose in trachea, Y-piece, both
280 bronchus & compartments) was in accordance with most of clinical data’s obtained *in vivo*, in healthy
281 volunteers or in patients during inhalation experiments with jet nebulizer devices (Rau, 2005;
282 Dugermier, 2016). This suggests that this new *in vitro* model presents a good level of *in vivo*
283 representativeness. However, the main limitation of the mechanical model lies in the fact that it is not
284 clear whether a separate low compliance lung compartment would act the same as isolated low
285 compliance hotspots embedded in otherwise normal lung parenchyma.

286 Our experiments support that heterogeneous reductions in lung compliance could play a role in the
287 distribution of aerosol penetration in fibrotic lung disease. Indeed, we observed both increased
288 aerosol penetration in the normal-compliance compartment, and reduced aerosol penetration in the
289 reduced-compliance compartment. Although the clinical relevance of this finding remains to be
290 defined, preferential deposition of inhaled drugs to preserved lung regions may result in a reduced
291 therapeutic index and a requirement for higher doses. There is thus a need for inhaled drug delivery
292 methods allowing to increase aerosol penetration and deposition in fibrotic, low compliance lung
293 regions.

294 The requirement to deliver drugs to low compliance regions of the respiratory system led to the
295 discovery that audible acoustic waves enhance deposition of nasally-delivered aerosolized drugs into
296 maxillary sinuses (Möller et al. 2014). By analogy, we hypothesized that acoustic/pressure waves may
297 increase aerosol penetration in the fibrotic lung model. Interestingly, although audible acoustic

298 waves had no effect, contrary to sinus models, low-frequency IPV pulses had a marked effect and
299 increased aerosol penetration 3-fold. Although the high variance in total mass balance mandates
300 caution in the interpretation of aerosol deposition experiments, the effect of IPV on aerosol
301 deposition in the experimental compartment was highly reproducible. This result is in line with the
302 observation by others that IPV increases penetration of therapeutic aerosols in a single compartment
303 mechanical model of the respiratory system, independent of model compliance (Karashima et al.
304 2019).

305 The mechanisms by which pressure pulses, such as those delivered by IPV, increase aerosol
306 penetration remain unknown. These mechanisms can be discussed by analogy with acoustic wave-
307 enhanced delivery of aerosols from the nose to maxillary sinuses, which, like distal airspaces in
308 fibrotic lungs, can be considered a low compliance cavity. A hypothesis is periodic compression of air
309 by acoustic waves, generating bidirectional airflow (Xi et al. 2017). Under this hypothesis, a maximal
310 effect is expected when transmission of the input pressure to the target cavity is maximal, that is,
311 when the frequency of acoustic waves equals the resonance frequency of the target cavity. Indeed,
312 matching soundwave frequency to resonance frequency of the maxillary sinuses (estimated with the
313 Helmholtz resonator model) increases aerosol delivery (Leclerc et al. 2015; Durand et al. 2012), while
314 frequency sweeps bracketing the estimated resonance frequency of the sinus yield optimal results
315 (Moghadam et al. 2018). Thus, we used the Helmholtz resonator model to estimate the resonance
316 frequency of fibrotic lungs.

317 The Helmholtz resonator is defined by the volume of its cavity (V), and the length (l) and area (a) of
318 the tube that connects it to outside air. The resonance frequency of the system can be calculated
319 from V , l (Durand et al. 2012). When using this model to describe fibrotic lung regions in IPF, we
320 assumed V to be within 1 and 3 liters, l as equal to 27 cm based on the sum of the average lengths of
321 the upper airways (7 cm) and of the bronchial tree down to segmental bronchi (20 cm), and a as
322 equal to 1.65 cm² based on 1) morphometric data of normal lungs (Crystal, West, and Weibel, n.d.),
323 2) the 1.32 increase in airway volume observed in IPF (Plantier et al. 2016) and 3) the assumption
324 that fibrotic lung regions comprise half of the lungs. These assumptions lead to estimation that the
325 resonance frequency of fibrotic lung may range from 23.8 Hz to 41.3 Hz.

326 At variance with the hypothesis that acoustic waves emitted at the resonance frequency of the target
327 may increase aerosol penetration, we observed that audible acoustic waves had no effect. Rather,
328 increased aerosol penetration was observed using low-frequency (≤ 3 Hz) IPV. Whether it is important
329 that acoustic/pressure pulse frequency is inferior to the resonance frequency of the system (here, 5
330 Hz) remains to be explored. Interestingly, in a 2-bottle physical model, maximal aerosol penetration
331 aerosol is observed with 100 Hz acoustic waves, while the estimated resonance frequency of the
332 model cavity is 303 Hz (Xi et al. 2017). The mechanisms contributing to IPV-increased aerosol

333 penetration thus remain to fully elucidate. Acoustic radiation pressure, which is well described in the
334 ultrasonic frequency range (Foresti et al. 2013) and is also physically relevant for low-frequency
335 (trending towards 0 Hz) acoustic waves (Löfstedt and Putterman 1991), may be a candidate
336 mechanism. Importantly, it is unclear how the frequency of the pressure waves that dissipate in the
337 airway relates to the frequency IPV volume/pressure pulses.

338 There is currently great interest in the development of inhaled therapies for IPF and other ILD.
339 Inhaled antifibrotic treatments would represent a key advance in IPF. Although several inhaled drugs
340 were or are currently being evaluated in clinical trials in IPF, such as glutathione (Borok et al. 1991),
341 heparin (Markart et al. 2010), interferon- γ (Diaz et al. 2012) and pirfenidone (Khoo et al. 2020), none
342 has been successful so far.

343 IPV is a respiratory physiotherapy technique that consists in delivery of small volumes of air into the
344 airways, typically in the 1-10Hz frequency and 10-50 cmH₂O pressure ranges (Reychler et al. 2018). It
345 is currently used to mobilize secretions, promote lung recruitment and improve gas exchange in
346 chronic conditions such as neuromuscular diseases, cystic fibrosis, and chronic obstructive
347 pulmonary disease. Although previous studies into IPV-coupled nebulized delivery were
348 disappointing (Reychler et al. 2004), our results suggest that IPV may be repurposed to improve
349 aerosolized drug delivery in the clinic.

350 Whether low-frequency (≤ 3 Hz) IPV is a feasible option to improve aerosol delivery to patients with
351 IPF depends on a number of issues, including 1) whether it does improve aerosol delivery to low
352 compliance lung regions *in vivo*, 2) whether it can be tolerated by IPF patients and 3) whether it is
353 innocuous. Although IPV appears to be well tolerated at frequencies in the order of 4-6 Hz (Nava et
354 al. 2006; Paneroni et al. 2011), whether low-frequency is tolerated for the duration required for
355 nebulized drug delivery remains to explore. Since patient discomfort is associated with IPV pressure,
356 our observation that 10cmH₂O did increase aerosol penetration in the low-compliance compartment
357 suggests that this method may be usable in patients. Although IPV appears to be generally safe
358 (Paneroni et al. 2011), it is essential to assess whether IPV-associated volutrauma or barotrauma is
359 deleterious in IPF patients. Notably, whether IPV can induce IPF exacerbations will need to be ruled
360 out before clinical application. IPF exacerbations are life-threatening events which can be triggered
361 by medical procedures such as mechanical ventilation and lung surgery (Ryerson et al. 2015). Adverse
362 effects of VPIP are rare in healthy subjects and patients with obstructive lung disease, with cases of
363 pneumothorax and proximal airway obstruction attributed to handling error (Flurin et al. 2009).

364
365

366 **Conclusion**

367 Using a mechanical model of fibrotic lung disease, we observed that reduced compliance of target
368 lung regions is a critical determinant of aerosol penetration, and thus presumably of aerosol
369 deposition. This finding underscores the need for techniques specifically tailored to enhance delivery
370 of inhaled aerosols to low compliance regions of the lungs, if fibrotic lung disease is to be successfully
371 treated with inhaled therapies. Low-frequency (≤ 3 Hz) IPV pulses may represent an attractive option
372 to increase aerosol deposition in fibrotic lung regions.

373

374 **Conflicts of interest**

375 SLG is an employee of DTF medical. LV is currently an employee of Nemera.

376

377 **Authors' contribution**

378 LA, SLG, and MC conducted experiments and analyzed results. LV, FO, SLG, NHV, JP provided
379 scientific input into acoustic/pulse wave physics and contributed to experiment design. LP
380 coordinated the overall project, designed and analyzed experiments, and drafted the manuscript. All
381 authors reviewed and approved the manuscript.

382

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389 loan, and Resmed France for providing the IPV generator as a loan.

390

391 **Contribution to the field statement**

392 Inhaled drug delivery is a mainstay of treatment for obstructive lung disease but is not currently used
393 in the context of fibrotic interstitial lung disease, due to insufficient deposition of inhaled aerosols in
394 diseased lung regions. In this work, we report the development of a novel two-compartment
395 mechanical model of the respiratory system that allows the simulation of the heterogeneous

396 alterations of lung geometry (increased volume of conducting airways) and physiology (reduced
397 compliance) of fibrotic lungs. The model reproduced the reduced penetration of model aerosols
398 observed in man. The model was then used to demonstrate how low-frequency intrapulmonary
399 percussive ventilation, a physiotherapy technique used for airway clearance in chronic respiratory
400 disease, increased aerosol penetration in low compliance regions of the respiratory system.

401

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542

543 **Table 1.** Mass balance of aerosol deposition experiments. Deposition in the normal lung model
 544 (compliance of both compartments set at 100 ml/cmH₂O) was compared with the fibrotic lung model
 545 (compliance of the Experimental compartment set at 50 ml/cmH₂O) without IPV. Deposition in the
 546 fibrotic model with 1Hz-40cmH₂O IPV was compared with the fibrotic lung model without IPV.
 547 Aerosol was expressed as % of the dose loaded in the nebulizer.

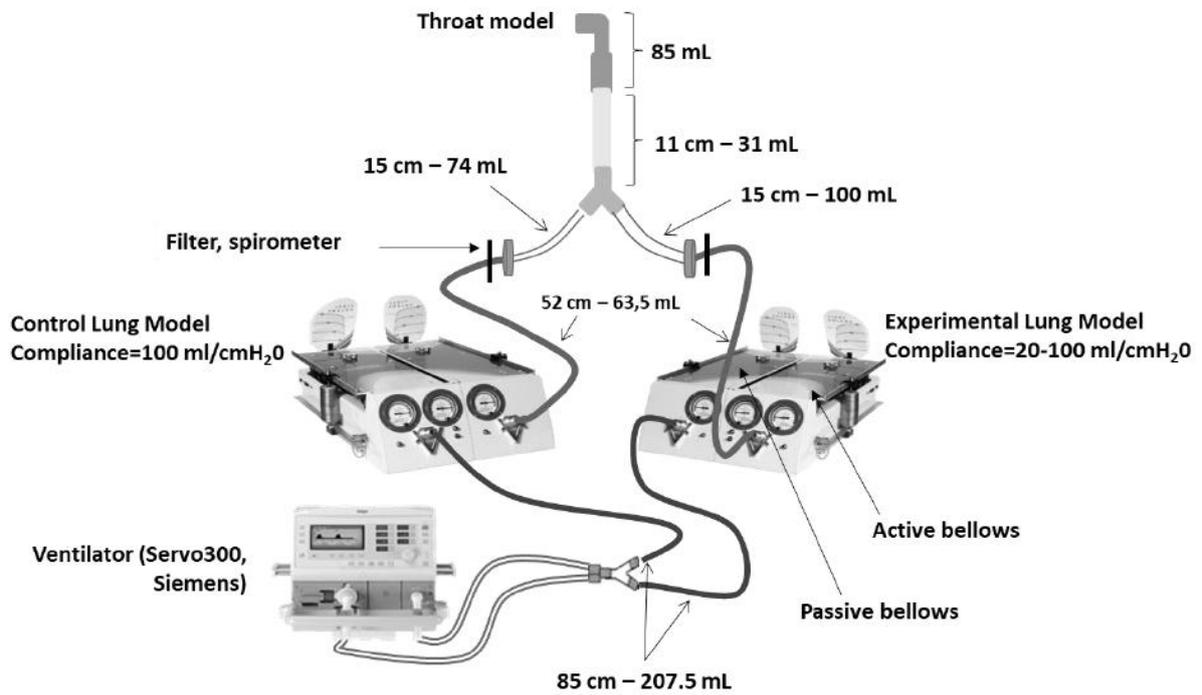
Deposition site	Normal lung model (n=3)	Fibrotic lung model, no IPV (n=6)		Fibrotic lung model with 1Hz-40cmH ₂ O IPV (n=6)	
	Deposition	Deposition	p value vs Normal	Deposition	p value versus Fibrotic
Expiratory filter	27,9 ± 6,7 %	32,2 ± 4,4 %	0.651	17,8 ± 5,3 %	0.001
Construct	7,1 ± 1,6 %	4,8 ± 1,5 %	0.530	14,1 ± 4,2 %	0.009
Nebulizer	50,7 ± 8,0 %	45,5 ± 5,9 %	0.896	49,1 ± 7,8 %	0.509
Alberta throat	1,7 ± 0,3 %	0,9 ± 0,3 %	0.009	4,0 ± 1,1 %	0.002
Trachea and Y-piece	0,6 ± 0,3 %	0,6 ± 0,3 %	0.910	0,5 ± 0,1 %	0.352
Control bronchus	0,4 ± 0,3 %	0,4 ± 0,1 %	0.580	0,3 ± 0,2 %	0.253
Control compartment	4,6 ± 1,2 %	5,5 ± 0,6 %	0.578	7,4 ± 1,9 %	1.000
Experimental bronchus	0,4 ± 0,3 %	0,2 ± 0,1 %	0.219	0,2 ± 0,2 %	0.079
Experimental compartment	4,4 ± 1,6 %	0,7 ± 0,2 %	0.020	2,2 ± 0,5 %	0.002
TOTAL	97,8 ± 12,1 %	90,7 ± 7,8 %	0.899	95,5 ± 17,2 %	0.626

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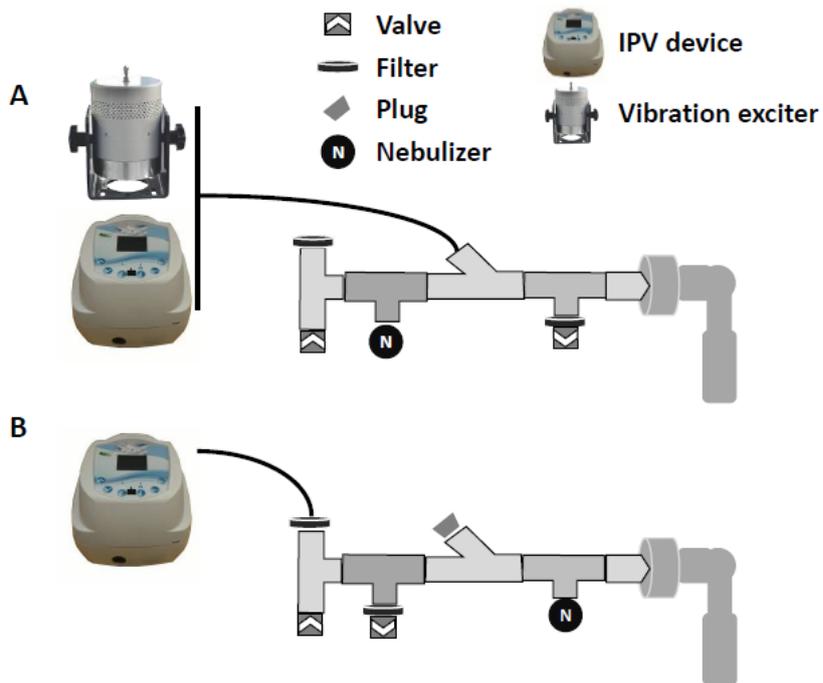
551 **Figure legends**



552

553 **Figure 1.** Schematic of the two-compartment model of the respiratory system used to simulate IPF
554 pathophysiology. The length and volume (measured) of corrugated plastic tubing used to model the
555 airways are featured.

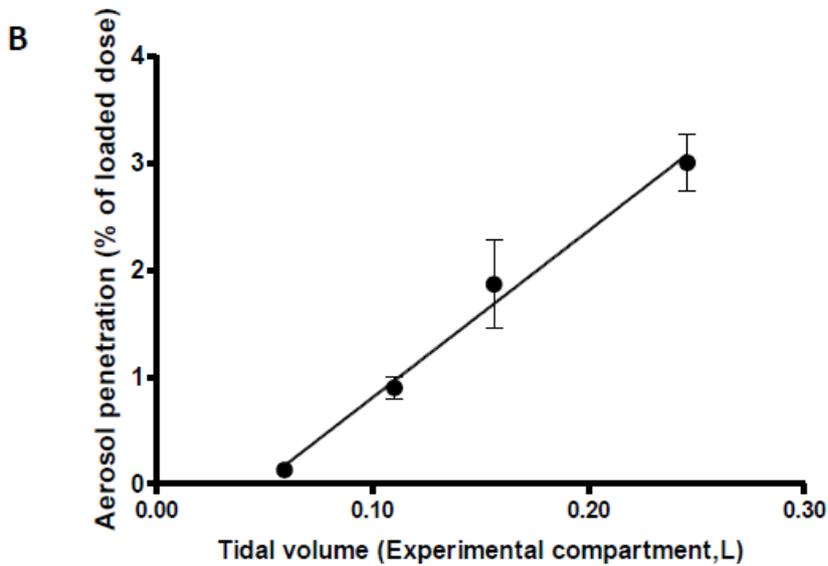
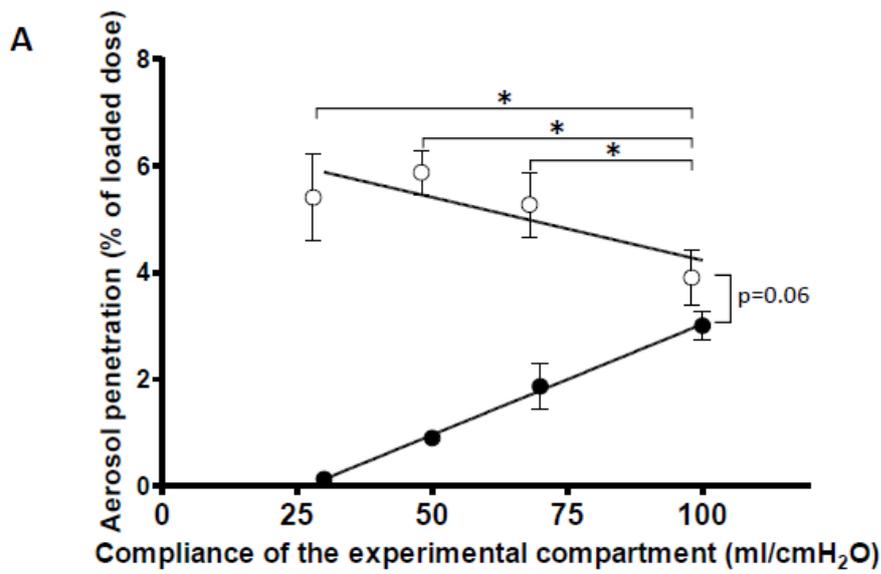
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557

558 **Figure 2.** Constructs used for acoustic/pulse pressure wave delivery during nebulization. All
 559 constructs are connected to the throat model (right). A: Construct used for acoustic wave delivery
 560 and initial IPV delivery. B: Construct used for multiple-frequency IPV delivery.

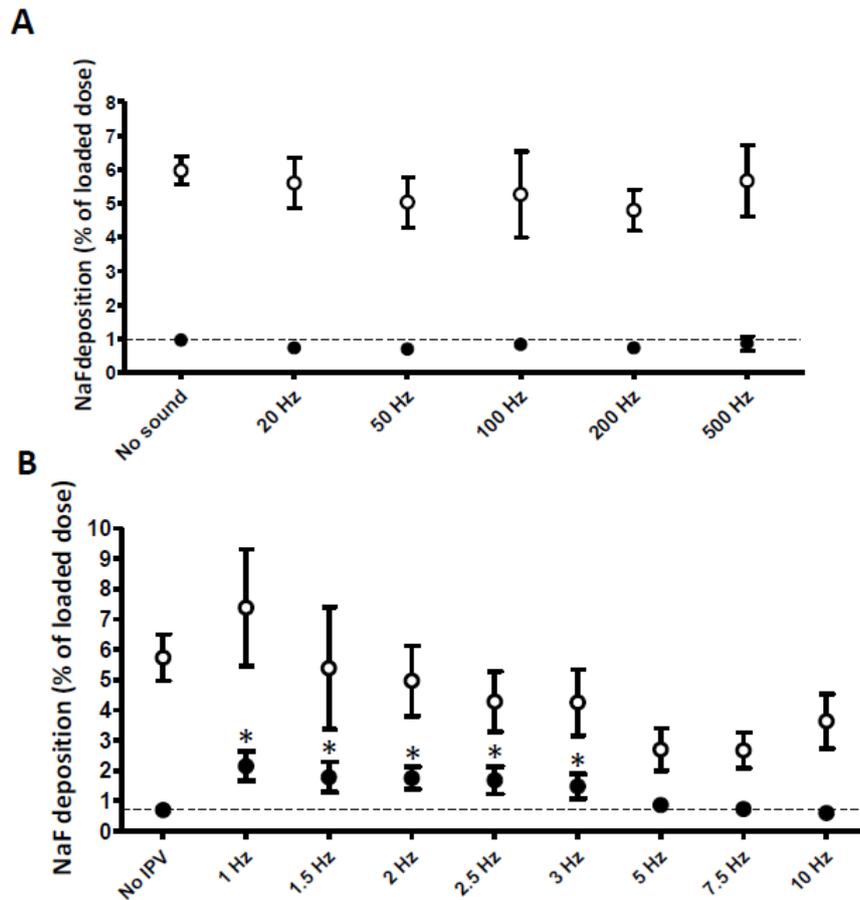
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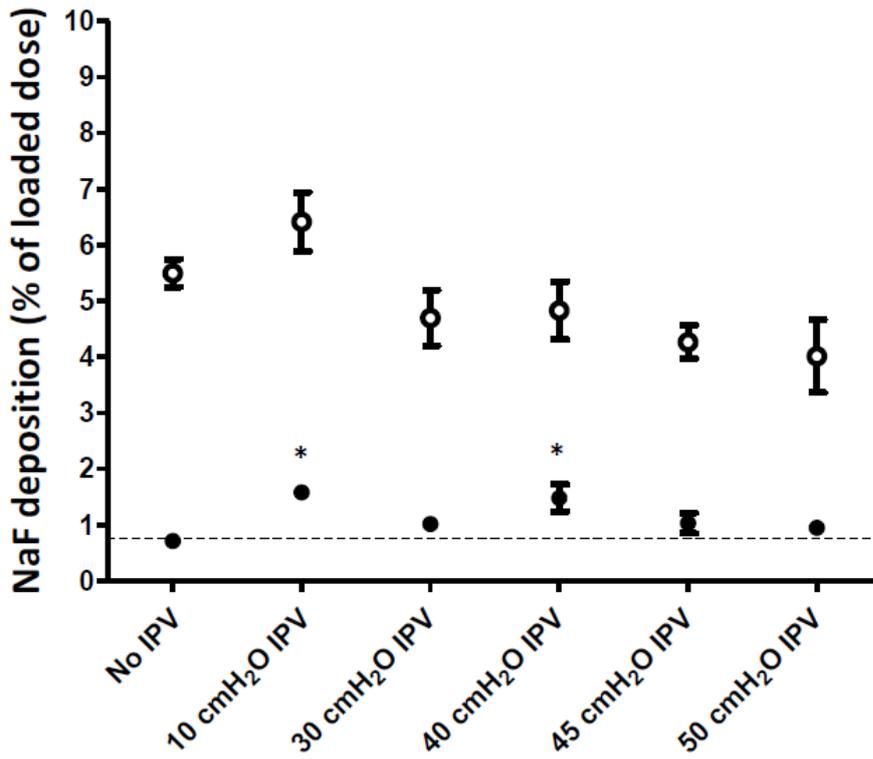
563 **Figure 3.** Aerosol deposition in the Control (open circles) and Experimental (filled circles) as a
 564 function of A: Compliance of the Experimental compartment and B: Tidal volume in the Experimental
 565 compartment. Compliance was set at 100 ml/cmH₂O in the Control compartment). *: p<0.05.
 566 Regression lines are shown. N=3. Means and SD.

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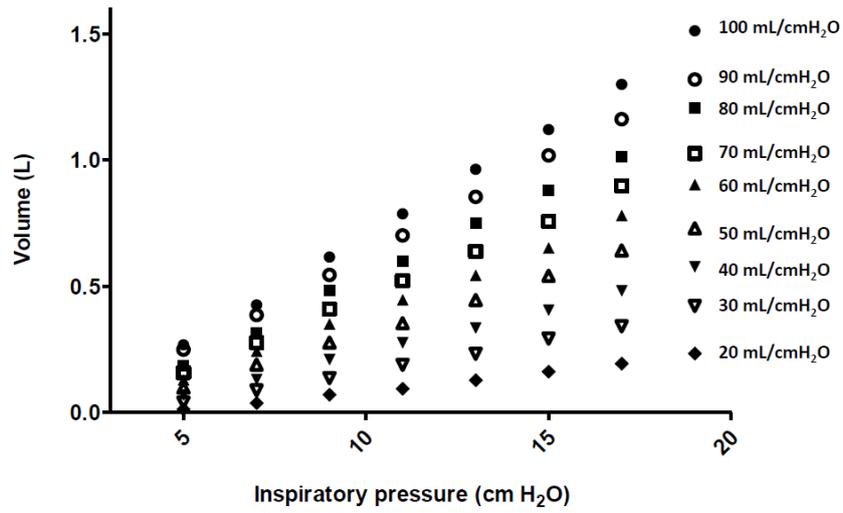
569 **Figure 4.** Aerosol deposition in the Control (100 ml/cmH₂O compliance, open circles) and
 570 Experimental (50 ml/cmH₂O compliance, filled circles) compartments of the model. A: 20-500 Hz
 571 audible acoustic waves (n=3) and B: 1-10 Hz IPV pressure pulses (n=6) were delivered during
 572 nebulization. Dotted lines show the control condition (no sound or no IPV). *: p<0.05 versus no IPV.
 573 Means and SD.



574

575 **Figure 5.** Aerosol deposition in the Control (100 ml/cmH₂O compliance, open circles) and
 576 Experimental (50 ml/cmH₂O compliance, filled circles) compartments of the model. Aerosol was
 577 delivered with either no IPV or 1 Hz IPV at different pressure levels. *: p<0.05 vs no IPV.

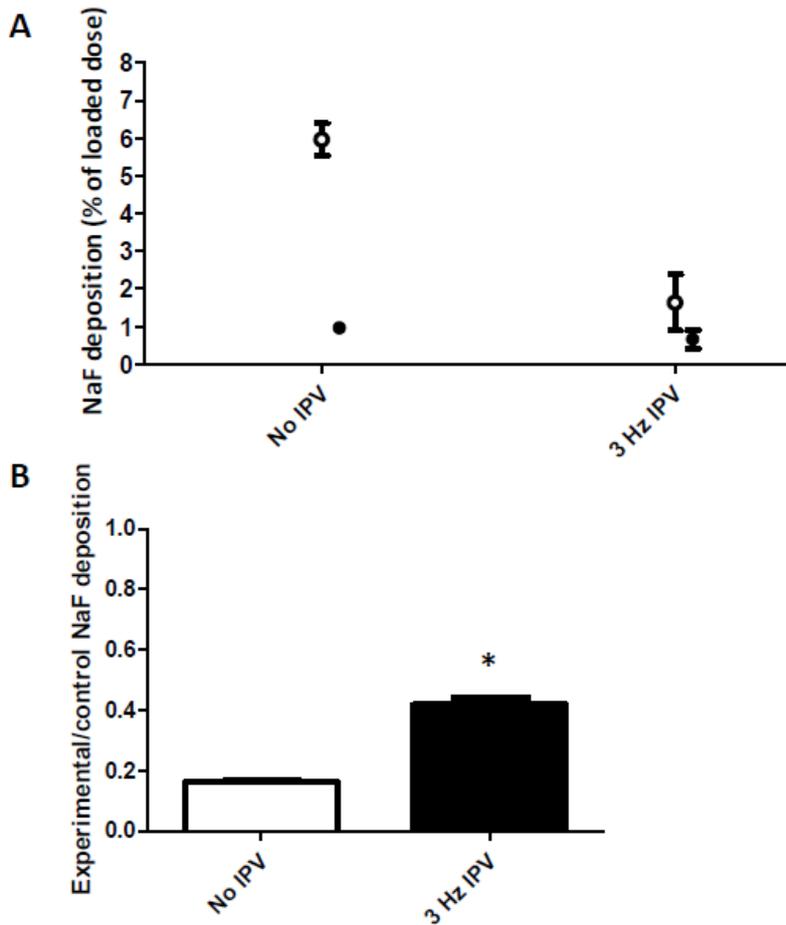
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579

580 **Supplementary Figure 1.** Tidal volume in the active bellows, as a function of inspiratory pressure in
 581 the passive bellows and set compliance. Means of 5 measurements are shown. Standard deviations
 582 were close to zero and are not shown.

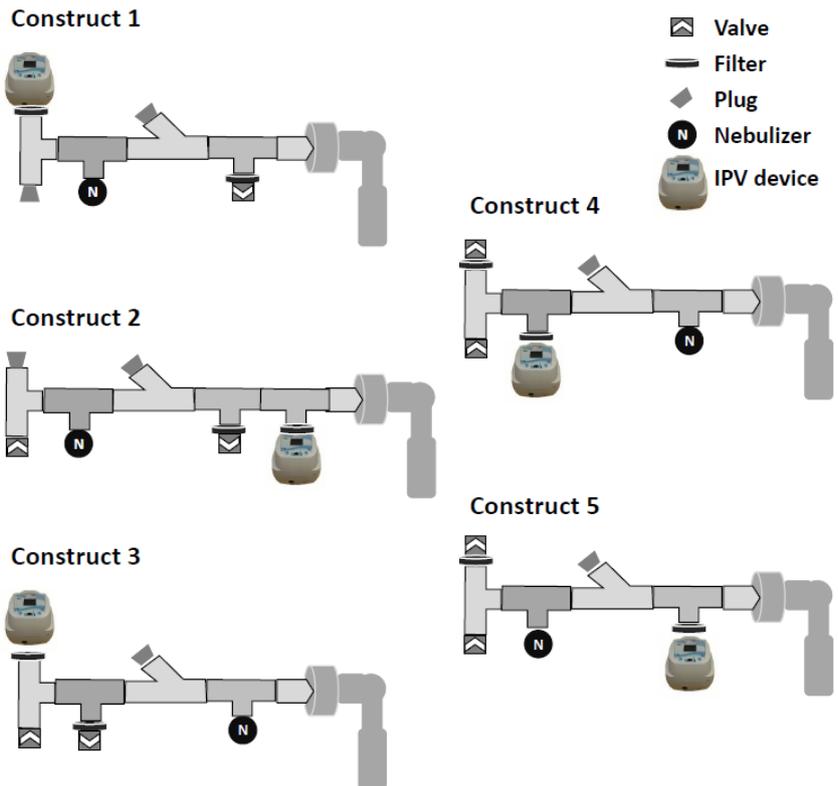
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584

585 **Supplementary Figure 2.** A: Aerosol deposition in the Control (100 ml/cmH₂O compliance, open
 586 circles) and Experimental (50 ml/cmH₂O compliance, filled circles) compartments of the model. B:
 587 Ratio of aerosol deposition in the Experimental and Control compartment. 3 Hz IPV was delivered
 588 using the nebulizer construct shown in Figure 2A. *: p<0.05.

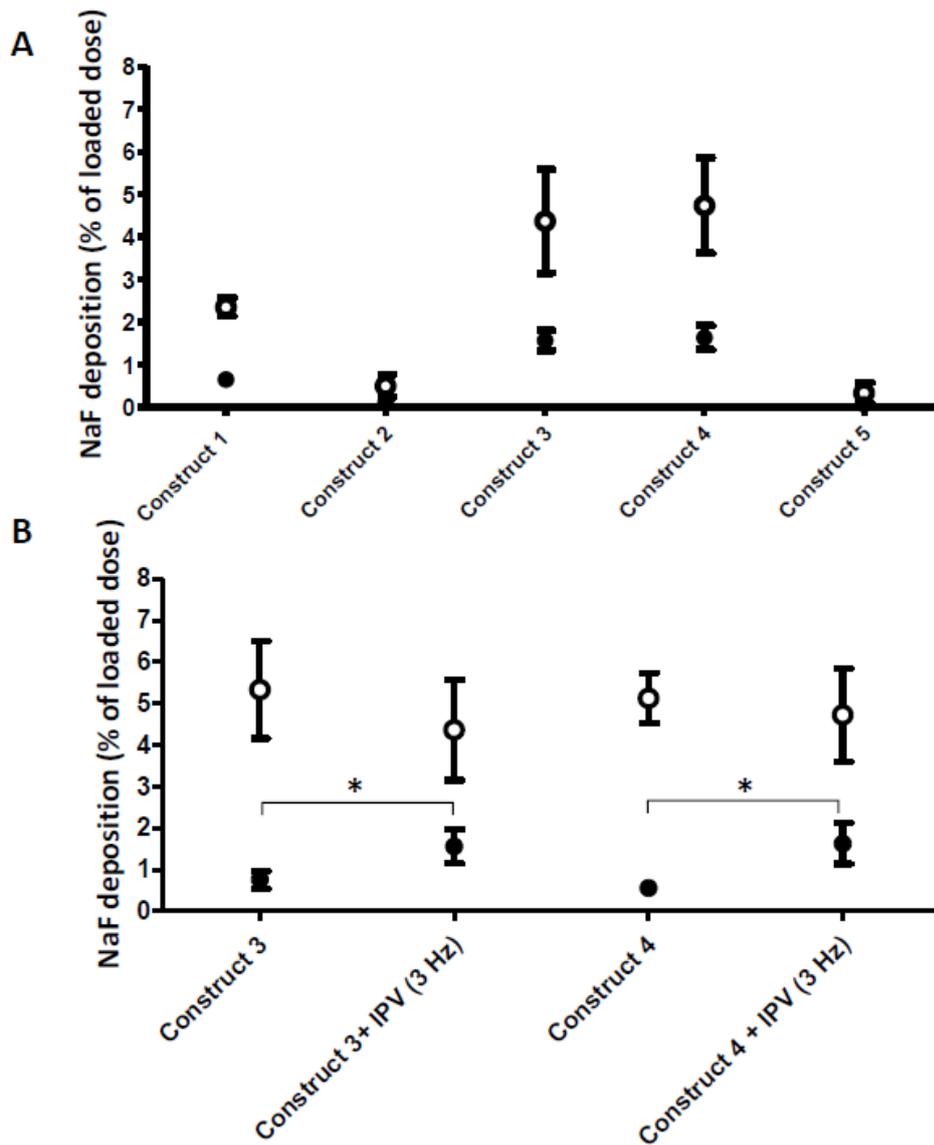
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590

591 **Supplementary Figure 3.** Nebulizer construct tested for IPV-enhancer aerosol deposition
 592 experiments.

593



594

595 **Supplementary Figure 4.** Aerosol deposition in the Control (100 ml/cmH₂O compliance, open circles)
 596 and Experimental (50 ml/cmH₂O compliance, filled circles) compartments of the model. A : IPV (3 Hz,
 597 40 cmH₂O) was delivered during nebulization, using the 5 constructs shown in Supplementary Figure
 598 S3. B : Constructs 3 and 4 were used either without or with IPV (3 Hz, 40 cmH₂O).

599