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EDITORIAL

Alveolar permeability and stretch: too far, too fast

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Mechanical ventilation is the most widely used therapy in intensive care units and the cornerstone treatment for patients with acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) [1]. Although essential for successful management of respiratory failure, mechanical ventilation can also exacerbate or even directly injure the lungs [2, 3]. This damage, commonly referred to as ventilator-induced lung injury (VILI), is characterised by increased permeability of the alveolar epithelium to fluids and macromolecules [4]. Mechanisms leading to such increase include direct mechanical damage caused by excessive stretch of the lungs (barotrauma and volutrauma) or by repetitive opening and closing of recruitable alveolar units (atelectrauma) [3]. In addition, mechanical stretch alone can trigger mechanotransduction pathways that may lead to local [5] and systemic inflammatory responses (biotrauma) [6].

To understand each of these mechanisms and their relative contribution to the pathogenesis of VILI, several *in vitro* strategies have been adopted. One of these strategies is to subject cells cultured on deformable substrates to stretch patterns mimicking those experienced by lung cells during mechanical ventilation [7–9]. This approach has provided a wealth of knowledge regarding the biological consequences of overstretching lung cells. It is well known, for instance, that large stretches induce: apoptosis, the release of inflammatory cytokines, structural changes in tight junctions, and plasma membrane breaks [3, 8–10]. A major drawback of *in vitro* stretch systems, however, is that the final readout is generally limited to biochemical markers. Stretch-induced synthesis, secretion and (de)phosphorylation of proteins as well as levels of mRNA production have become routinely available, but a quantitative analysis of microstructural changes in the epithelium and their influence on epithelial permeability has remained virtually inaccessible.

To overcome this limitation, CAVANAUGH *et al.* [11] recently developed a technique to measure the permeability of cell monolayers subjected to stretch. The technique is based on growing cells on a substrate that is both stretchable and porous. Given that uncharged micromolecules can freely diffuse through the substrate pores, the permeability of the overlying cell monolayer can be quantified. CAVANAUGH *et al.* [11] used this technique to show that high amplitude stretch (analogous to high tidal volume) leads to increased alveolar epithelial permeability. In the current issue of the *European Respiratory Journal* (*ERJ*), COHEN *et al.* [12] focus on the role of stretch frequency (analogous to ventilation rate) and amplitude offset (analogous to positive end-expiratory pressure (PEEP)).

The role of stretch frequency in VILI is highly pertinent but often overshadowed by the role of stretch amplitude. Indeed, whilst there are hundreds of studies focusing on the effect of large tidal volumes on lung injury, only a handful have addressed the role of ventilation rate. The studies that do address this almost uniformly suggest that high ventilation rates play an injurious role in VILI. At the animal level, for example, high ventilation rates have been associated with increases in the volume of epithelial lining fluid, in phosphorylation levels of Akt and extracellular signal-regulated kinase 1/2, in capillary permeability, in lactate dehydrogenase activity, and in the total mass of protein in bronchoalveolar lavage, including cytokines such as interleukin-6 and tumour necrosis factor-α [13–15]. At the cell culture level, increasing stretch frequency has been shown to induce plasma membrane breaks and cell death [8, 9]. In the current issue of the *ERJ*, COHEN *et al.* [12] provide further evidence supporting the harmful effect of high stretch frequency by showing that it causes increased permeability of the otherwise healthy alveolar epithelium. This evidence contrasts with the relatively tolerant ARDS network protocol that allowed respiratory rates up to 35 breaths·min^{-1} to maintain the pH >7.3 [2]. Interestingly, COHEN *et al.* [12] found that the injurious effect of increasing stretch frequency disappeared if stretch amplitudes were kept small. This finding is consistent with the observation that strategies based on high ventilation rates but small tidal volumes are safe [16].

A second point addressed by COHEN *et al.* [12] is the relationship between PEEP and alveolar permeability. While the clinical community increasingly agrees on the beneficial effects of PEEP, the optimal strategy for its use remains controversial and lacks mechanistic basis from *in vitro* studies. To mimic PEEP, COHEN *et al.* [12] added a tonic component (a constant strain offset) to the oscillatory component of the stretch signal. After studying various combinations of the oscillatory and tonic components, they found that changes in epithelial permeability were not related to the magnitude of the tonic component. Instead, the key determinant of alveolar permeability was peak amplitude. When extrapolated to the *in vivo* context, this finding suggests that the level of PEEP does not harm the alveolar epithelium as long as the oscillatory component is controlled to keep the peak volume within a protective range.

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It should be noted that the experimental approach of COHEN *et al.* [12] does not include several injurious factors that may act synergistically with stretch during the development of VILI. A number of these factors, including sepsis [17], hypoxia [18], hyperoxia [19], hypercapnia [20], and the presence of proteases [21], have already been shown to interact with stretch. In addition, stretch alters the properties of the extracellular matrix that supports the alveolar epithelium, which could also regulate alveolar permeability during overdistention [22, 23]. Extrapolation of the findings from the study by COHEN *et al.* [12] to the complex pathological environment of ALI and ARDS is thus unclear. Nonetheless, the advantage of their experimental approach is its ability to isolate the mechanical insult from other injurious factors, thus enabling a better mechanistic understanding of the pathogenesis of VILI.

What do these findings tell us about mechanism? VLAHAKIS *et al.* [9] provided substantial evidence that overstretching the alveoli causes epithelial injury through rupture of the plasma membrane. When subjected to stretch of physiological magnitude, the cell membrane accommodates area changes by means of unfolding, stretching and lipid trafficking. At large strain magnitudes and rates, however, these mechanisms of membrane mechanoprotection are overwhelmed and the plasma membrane is ruptured, ultimately resulting in cell death and paracellular gap formation. While this mechanism is likely to contribute to VILI, COHEN *et al.* [12] found that the threshold of increased permeability is lower than that of cell death, suggesting that other mechanisms should come into play to explain stretch-induced permeability.

COHEN *et al.* [12] propose that one of these mechanisms may be paracellular gap formation owing to the reorganisation of the actin cytoskeleton (CSK) and junctional protein complexes. The fact that mechanical stretch alters CSK dynamics and adhesion is now well documented [24] but its dependence on stretch frequency has yet to be determined. A second mechanism may lie in the stress that the CSK exerts on cell–cell junctions. If the CSK were a standard linear material, such as a spring or a rubber band, then the tension that cell–cell adhesions would need to withstand during stretch would increase proportionally to the magnitude of the applied strain. This is not the case, however. Due to the semiflexible nature of most of its polymers, stresses within the cell increase with strain in a roughly quadratic manner. Such nonlinear dependence dramatically increases the cell–cell junctions' propensity to fail when subjected to large strain [21, 25]. In addition, cell stiffness also increases with frequency, which may bring cell–cell junctions even closer to rupture at high frequencies. The combination of these two mechanical properties of the CSK could lie at the origin of paracellular gap formation when the epithelium is stretched too far and too fast.

In addition to mechanisms of injury of a purely biophysical origin, increased permeability could also arise from the activation of mechanotransduction pathways [3, 6]. These pathways are triggered by force-induced exposure of cryptic binding sites, by the opening of stretch-activated ion channels and by the strengthening of receptor–ligand interactions during stretch [26]. Such mechanisms are believed to be the origin of inflammatory responses to stretch, including cytokine release and production of reactive oxygen species [3], but their mechanistic connection to alveolar permeability is unclear.

The study of COHEN *et al.* [12] leaves a major question unanswered: how does stretch with high peak amplitude and high frequency interact with other deleterious factors associated with acute respiratory distress syndrome and acute lung injury to aggravate epithelial permeability? This question constitutes a new avenue of basic research in ventilation-induced lung injury that can be tackled one step at a time using the approach of COHEN *et al.* [12]. Meanwhile, the research community is left with further evidence that lowering the rate at which alveolar epithelial cells are stretched may play a protective role in ventilation-induced lung injury. This notion should be kept in mind in the open debate concerning the optimal ventilation strategies for patients with acute lung injury and acute respiratory distress syndrome.

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