# CHANGES IN ALVEOLAR EPITHELIAL CELL PLASMA MEMBRANE SURFACE AREA WITH STATIC STRETCH

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## INTRODUCTION

Alveolar epithelial cells are subject to cyclic stretch throughout a lifetime of breathing with no apparent harm. During mechanical ventilation, however, injured or diseased lungs tend to suffer from regional overinflation, which translates to injuriously high cyclic strain on alveolar epithelial cells. In order to reduce the clinical incidence of ventilator-induced lung injury (VILI), investigators are trying to determine how alveolar epithelial cells sense stretch and respond to different stretch modes and amplitudes, and how beneficial responses to stretch can be enhanced, while harmful responses are avoided.

In previous studies, we have shown that alveolar epithelial cells are much less sensitive to static stretch than to cyclic stretch [1,2]. We have also found that cells subjected to cyclic stretch superimposed over a static stretch baseline respond only in proportion to the cyclic component [2]. Finally, we have found that stretching and holding cells for a period of time inures them against cell death during subsequent cyclic stretch [3]. In sum these observations suggest that alveolar epithelial cells held statically in a stretched position remodel themselves in a way that renders the stretch stimulus less perceptible. It is our hypothesis that this remodeling occurs primarily through changes in shape and volume of the plasma membrane (PM).

While there are few articles in the literature on lipid trafficking/ cell membrane expansion in epithelial cells, studies of lipid trafficking and surface area change in neuronal cells abound. Investigators have found that lipid vesicles traffic to the cell surface (and are later reabsorbed) not only for neurotransmitter release, but that lipid trafficking acts as a means of regulating neuronal cell surface tension [4-6]. One study, which did study epithelial cells using fluorescent dyes, reported lipid trafficking and cell surface area in transformed A549 cells in response to stretch [7]. However, measurements based on dye intensity make quantitation difficult and may only show accelerated lipid recycling, not necessarily net increase in PM volume.

In this study we have used an alternative method, which measures change in PM surface area by measuring change in capacitance across it before and after stretch. In essence cell capacitance is determined by three factors: PM surface area, A; thickness, t; and a material dielectric constant, k, as shown in the equation: $C = k*A/t$. Because energetic constraints limit change in PM thickness [8], significant change in cell capacitance can be attributed primarily to change in PM surface area. Together with confocal microscopy studies of cells before and after stretch, these data let us distinguish between cell surface expansion via PM unfolding or net PM accumulation via lipid trafficking.

## METHODS

### Cell Culture

Alveolar epithelial cells were isolated from Sprague-Dawley rats and seeded on fibronectin-coated Silastic membranes using previously described procedures [2,9]. Cells were maintained at 37°C in 5% $CO_2$ in MEM supplemented with Earle's salts, 10% FCS and 25µg/ml gentamicin. Before stretch and electrophysiological analysis, cells were rinsed 3 times and left in DMEM without $NaHCO_3$, with 1% penicillin (1000U/ml)/streptomycin (10mg/ml) and 20mM HEPES.

### Laser Confocal Microscopy and Image Analysis

Cells were loaded with calcein AM for 30 min, and image slices were captured through the depth of the cell by confocal fluorescent microscopy (z-step = 0.25-0.3 µm). Cells were then stretched in a custom device (25% change in surface area, ΔSA, of the Silastic membrane), held for 10 min, and re-imaged in the stretched position. In each slice, cell cross sections were located with Scion Image (Scion Corporation) using a built-in particle analysis algorithm. From these image stacks, 3-dimensional virtual cells were reconstructed and volume and apparent cell surface area (CSA) were calculated for each cell in the stretched and unstretched states. Comparisons were made between the change in 2-dimensional projected surface area (ΔPSA) and ΔCSA found with confocal microscopy.

### Capacitance Measures

Wells were mounted on a microscope stage in the stretching device. Glass micropipettes were pulled to a final resistance of 4-6 MΩ, loaded with a solution of (in mM): 156 KCl, 1.5 $CeCl_2$, 1 $MgCl_2$, 10 HEPES, 1 EGTA, pH 7.3, and mounted over a silver electrode. A

saturated salt agar bridge placed in the extracellular medium served as a reference electrode. Electrophysiological measurements were made with an EPC9 amplifier and the companion Pulse software (HEKA Electronik, Lambrecht, Germany).

Using a micromanipulator, the micropipette was brought into contact with the cell membrane, and once a seal formed, the membrane was breached to allow whole cell recording. Pipette capacitance was automatically compensated, and electrode potential was set at of -60 mV. Series resistance and whole cell capacitance were recorded. Later, cells were stretched (25% ΔSA for Silastic membrane), held in the stretched position for at least 5 minutes and probed using the above technique. Images were captured of all stretched and unstretched probed cells and used for cell area to capacitance comparisons.

## RESULTS

### Projected surface area vs. total cell surface area

In cells stretched between 3%-25% ΔPSA, the percent ΔCSA correlated closely with percent ΔPSA (Figure 1). This near 1:1 match, however, does not necessarily mean that stretched cells expand isometrically, but rather that the CSA of a broad, thin cell depends strongly on the area of its base.

Capacitance also increased in cells that were stretched and held at 25%ΔPSA (n=7) compared to unstretched cells (n=6), indicating lipid trafficking (Figure 2). If stretched cells expanded their surface area simply by PM unfolding, one would expect the stretched cell data to shift to the right with no increase in capacitance. Because the capacitance-PSA relationship is not significantly different between stretched and unstretched cells, we conclude that within 5 minutes of static stretch, cell surface area increased via lipid trafficking and a net increase PM volume rather than by membrane unfolding.

## DISCUSSION

Recently Vlahakis et al. demonstrated qualitatively that lipid trafficking occurs within 90s of static stretch in alveolar epithelial cells. In this study, within 5 min of static stretch, we measure that sufficient lipid trafficking has occurred to make the capacitance of a stretched cell no different statistically from that of an unstretched cell with the same PSA. Such PM remodeling, as proposed in our hypothesis, would certainly account for our earlier observation that static stretch may condition cells against cyclic stretch injury.

PM remodeling during static stretch may provide broader insights for VILI as well. Many alveolar epithelial responses to stretch have been linked to membrane-associated elements such as stretch-activated ion channels [2] and G-proteins [10], which respond to changes in PM tension. If, with static or sufficiently slow "quasi-static" stretch, PM remodeling could relieve cell tension, it might be possible to avoid undesirable stretch stimuli. While it would be unrealistic to slow mechanical ventilation too far below physiological rates, it could be possible to combine moderate tidal volumes at normal frequency with maneuvers such as low frequency shifts in positive end-expiratory pressure (PEEP) to recruit unventilated lung regions. By better understanding PM response to stretch, our goal is to suggest strategies such as this which might provide adequate ventilation while reducing or even circumventing VILI and ultimately to improve outcome for mechanically ventilated patients.

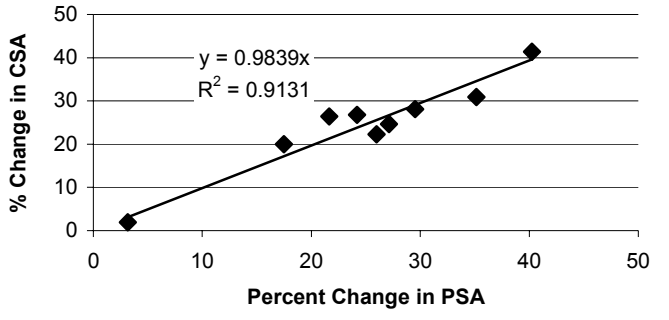


**Figure 1. Change in CSA as a function of change in PSA. Cells stretched within a physiological range exhibited a high correlation between in %ΔCSA and %ΔPSA, most likely due to alveolar epithelial cells' thin, flat shape.**

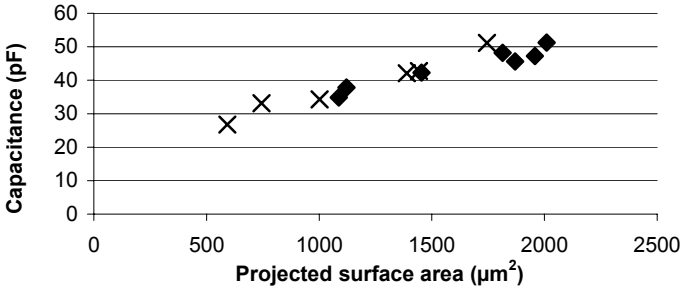


**Figure 2. Cell capacitance as a function of PSA. Capacitance increases with cell size. The similar relation for stretched (◆) and unstretched (X) cells shows that a stretched cell remodels its PM to appear as a larger, unstretched cell.**

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