Electronic Supplementary Material (ESI) for Integrative Biology.
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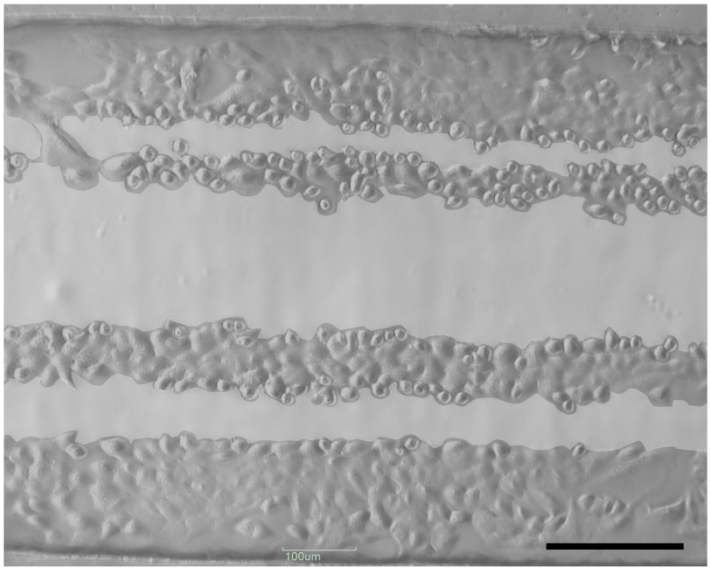
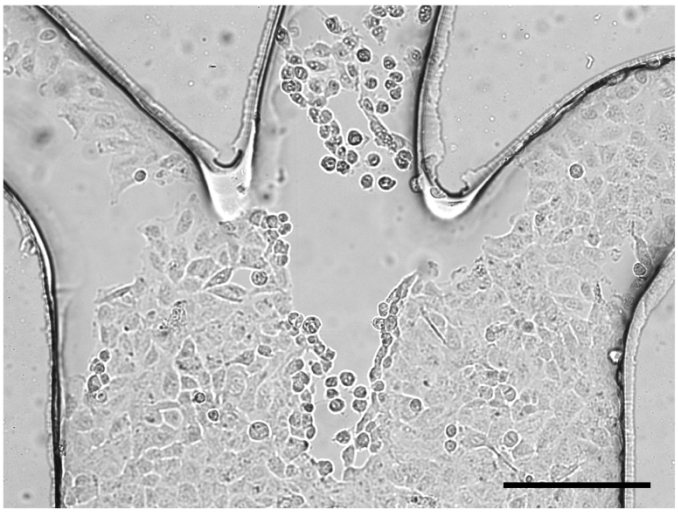
Concern: review of the article IB-ART-06-2014-000149 entitled “Potential of microfluidic lung epithelial wounding: towards in vivo-like alveolar microinjuries”

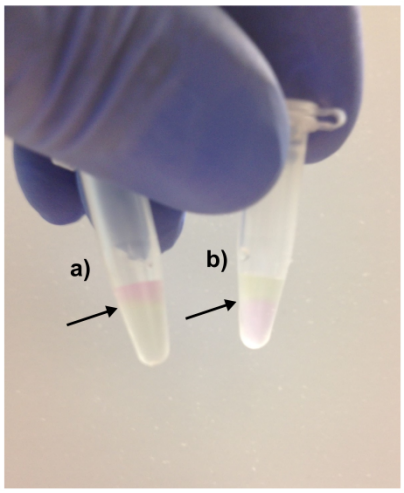
Revised Supplementary Material



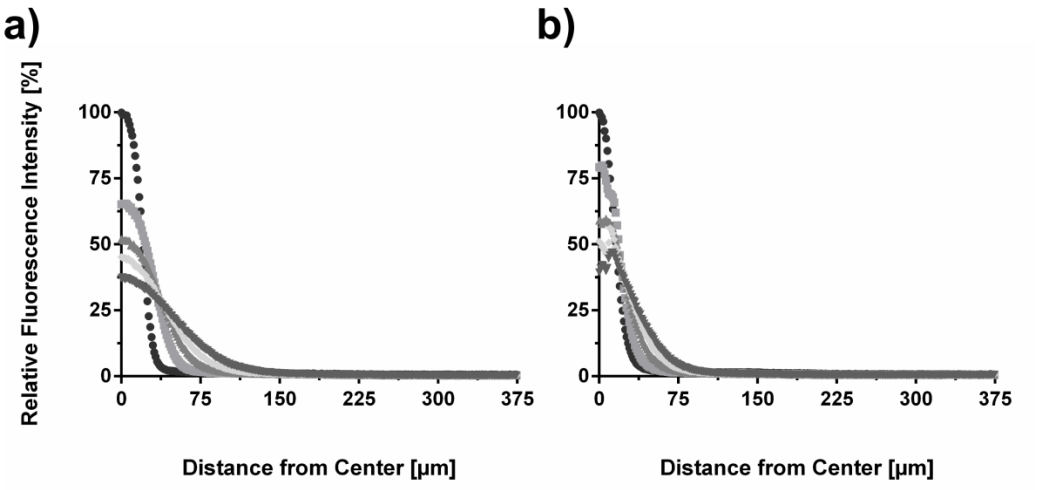
Suppl. Fig. 1: Parallel microwounds of different wound sizes in a monolayer of A549 cells. Hydrodynamic flow focusing was used to induce a central wound of 150µm and two lateral microwounds of 50µm or 25µm, respectively. Scale bar: 200µm



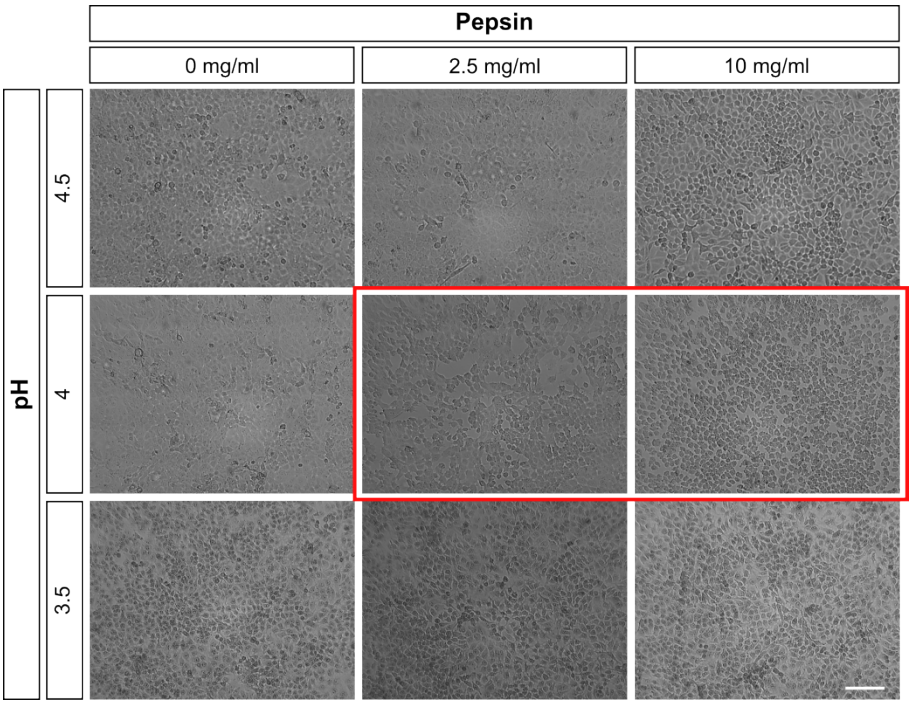
Suppl. Fig. 2: A549 cells exposed to a focused flow of 2.5 mg/ml pepsin in acidified serum-free medium (pH 4). In the absence of an aqueous two-phase system, wound formation stopped shortly after flow convergence at the channel opening. Scale bar: 200µm



Aqueous two-phase system using 5% (w/w) 500 kDa dextran (DEX) and 2.5% (w/w) 35 kDa polyethylenglycol (PEG) in serum-free RPMI 1640. Both phases, the lower DEX-rich and the upper PEG-rich phase, were then separated and acidified with hydrochloride acid (HCl) to pH 4. Due to the phenol red in the culture medium, acidification induced a distinctive color change. After pouring together the acidified DEX and the pH-neutral PEG-phase (a), or vice versa (b), both phases remained separated with clear separation lines (arrows).



Sodium-fluorescein diffusion during flow focusing using PBS (a) and an aqueous two-phase system (b). Sodium-Fluorescein was dissolved in PBS to a final concentration of 1 mg/ml and hydrodynamically focused in cell-free microfluidic platform with a ratio sheath-to-central flow of 1:30. The relative fluorescence intensity profile was then measured at various positions along the microchannel length. Similarly, sodiumfluorescein was hydrodynamically focused using an aqueous two-phase system of 5% (w/w) 500kDa dxtran and 2.5% (w/w) 35kDa polyethylenglycol in PBS. The positions represented in the graphs are 0.5 mm (●), 1.5 mm (■), 3.5 mm (▲), 5.5 mm (♦) and 9.5 mm (▼) after the channel opening. As the sodium tracer propagates along the microchannel, diffusive transport lead to reduced peak intensity in the center and spread intensity toward the periphery of the channel. The use of an aqueous two-phase system reduced the diffusive transport. Interestingly, sodium-fluorescein seems to accumulate in the interphase at approximately 20-25 µm from the center.



Suppl. Fig. 5: Type II alveolar epithelial-like (A549) cells exposed to various concentrations of hydrochloric acid (HCl) and pepsin. The addition of pepsin alone did not induce any morphologic changes in the cells. Contrary to that, the addition of HCl revealed pronounced nuclei condensation at pH < 4. Monolayer disruption was, however, only observed upon concomitant treatment with pepsin and a moderate level of HCl (highlighted). These findings suggest that the low pH of acidic microaspirations (pH < 4) directly damages the alveolar epithelium. Further, evidence is provided that pepsin from particulate or liquid refluxes cleaves cell anchoring proteins at non-harmful pH-levels. A synergistic action of pepsin and HCl may therefore cause severe epithelial damage during microaspirations. Scale bar: 100µm

Suppl. Video 1: Wounding with Trypsin/EDTA

Suppl. Video 2: Wounding with Pepsin/HCl