

Supplementary Information – Chandrasekaran et al., Magnetic microboats for floating, stiffness tunable, air liquid interface epithelial cultures

Supplementary Movie SM1, demonstrating magnetic submersion and release of an individual microboat

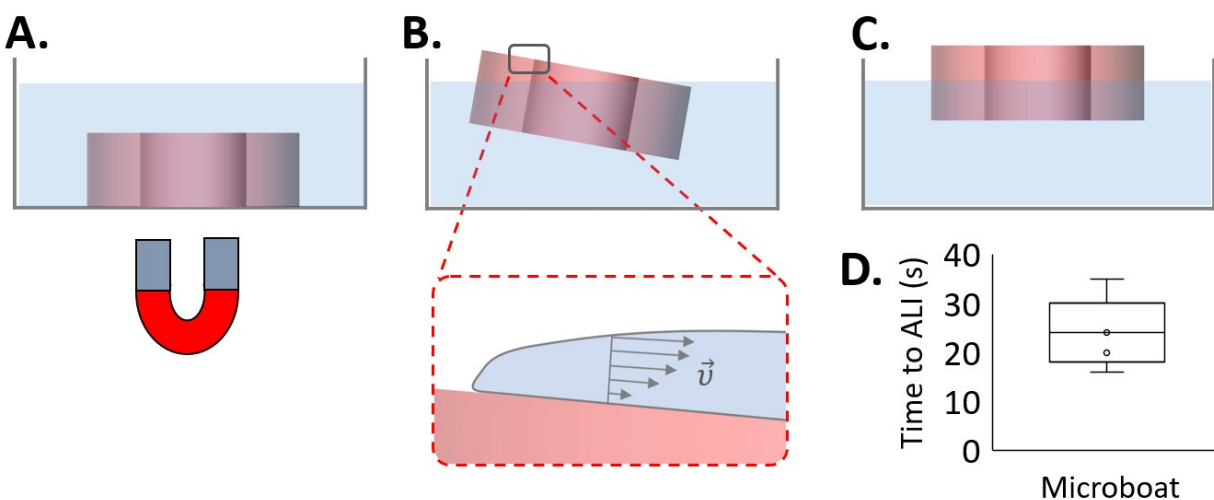


Figure S1. (A-C) Schematic representation of microboats rising to the surface and establishing an air-liquid interface. (B) When microboats are released, they breach the surface within one second of removing the magnetic field. (B-insert) The liquid then drains off the top surface in < 40 seconds to (C) establish an air-liquid interface. (D) Characterization of time required to form the air liquid interface after magnet release. The box plot represents median and interquartile range, while the whiskers indicate the range of measurements taken. All microboats tested established air-liquid interface within 40 seconds.

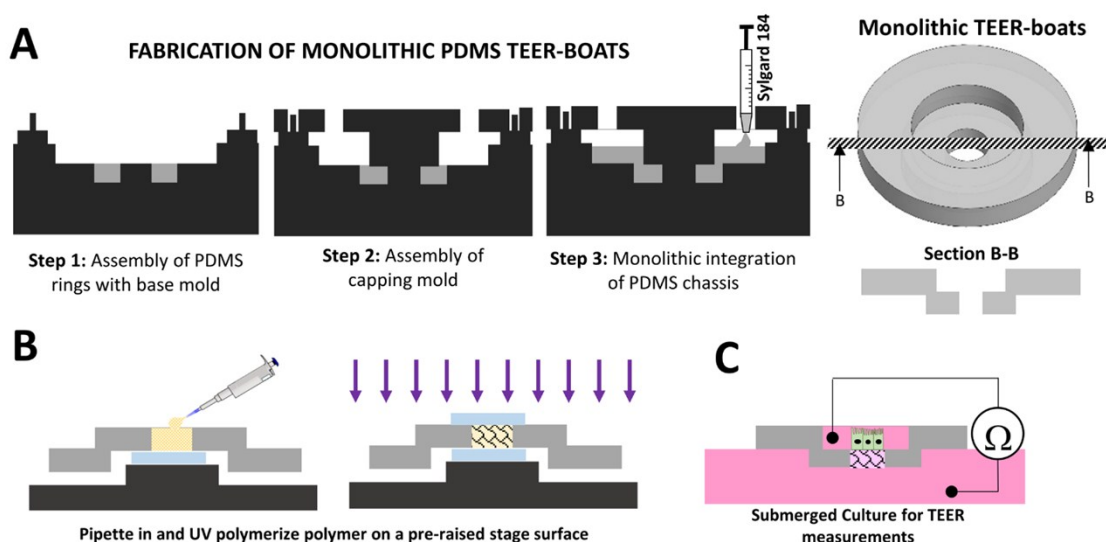


Figure S2. To fabricate TEER-facilitating devices, a base mold and a capping mold were 3D printed, to replicate features on both sides of the PDMS. The simple PDMS ring devices, were positioned in the base mold (schematic in Fig. 2B) and PDMS was cast on top of it. Using alignment pins, a capping mold was

positioned on top of the base mold, to create a concentric counterbore on the device. The PDMS devices were then cured and released from the disassembled molds.

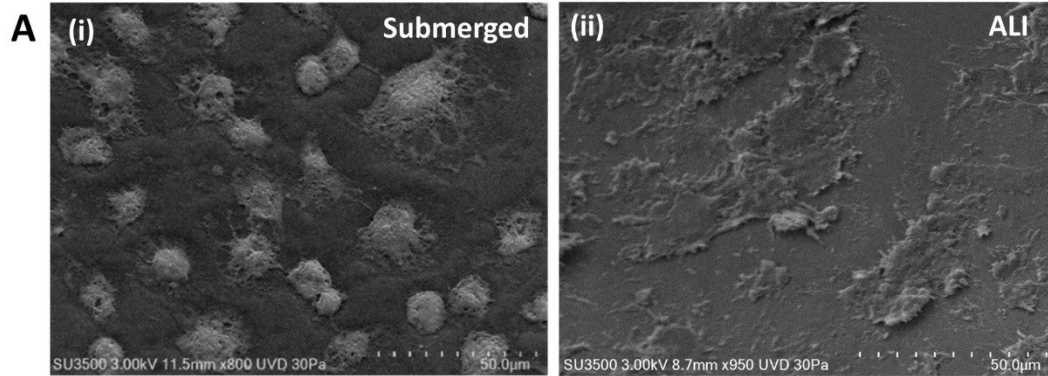


Figure S3. SEM image of sparsely-seeded human bronchial epithelial cells cultured in (a) submerged culture and (b) at Air Liquid Interface on the microboat devices demonstrates that cells exhibit a typical rounded morphology under submerged culture whereas they exhibit a well-spread morphology at ALI.

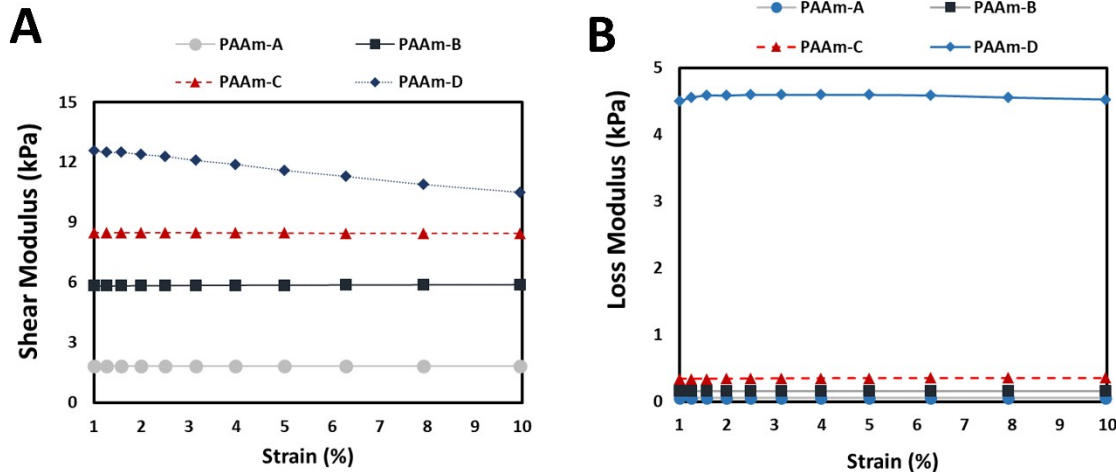


Figure S4. Mechanical characterization of (A) shear and (B) loss moduli in polyacrylamide formulations via shear rheometry. PAAm-D formulations showed strain-softening behaviour and a larger loss modulus. However, these effects are not substantial over the range of strains expected to be generated by the cells.

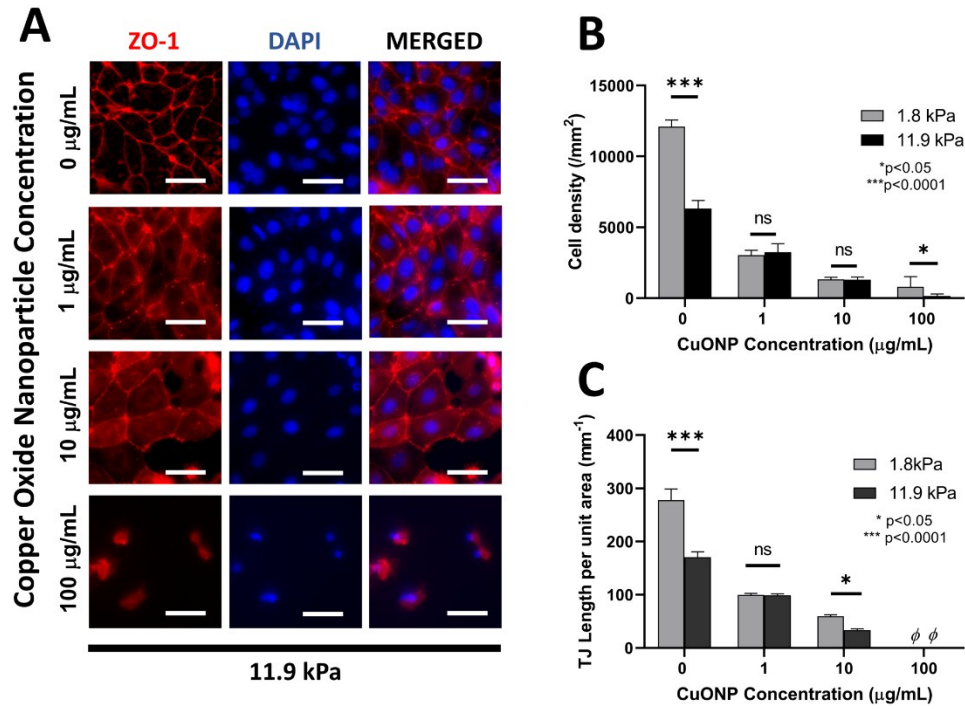


Figure S5. Both soft and stiff hydrogel matrices used in these studies allow sufficient diffusion through the hydrogel thickness to affect cell function, as evidenced by dose-dependent exposure responses to toxic copper oxide nanoparticles. (A) Fluorescent images of ALI-cultured epithelial cells on $G = 11.9$ kPa substrates after 24 hours of exposure to varying doses of Copper Oxide nanoparticles (CuONPs) in the underlying media. (red = ZO-1 tight junction protein; blue = nuclear DAPI; scale bar = $50 \mu\text{m}$). Cultures were quantified using (B) cell density and (C) tight junction length per unit area for 1.8 and 11.9 kPa substrates, to demonstrate morphological and functional differences arising from nanoparticle exposure (** $p < 0.01$, *** $p < 0.001$, n.s. $p > 0.05$ by two-tailed ANOVA with Tukey post-hoc comparisons; $n = 3$).