Fast-track development of an *in vitro* 3D lung/immune cell model to study *Aspergillus* infections

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Video Legends

Video 1 Comparison of cilia development and beating between NHBE cells grown in ALI under perfused vs. static conditions. (a) Perfused conditions were superior in ciliogenesis as well as their activities while ***(b)*** under static conditions NHBE cells showed single spots of cilia development and relatively slow beating.

Video 2 Mucociliary clearance and cilia activity analyzed by live cell microscopy. The surface of epithelial cells grown under perfusion was stained using wheat-germ-agglutinin (WGA, red) while intracellularly cells were stained using mitotracker for mitchondria (green).

Supplementary Figure Legends

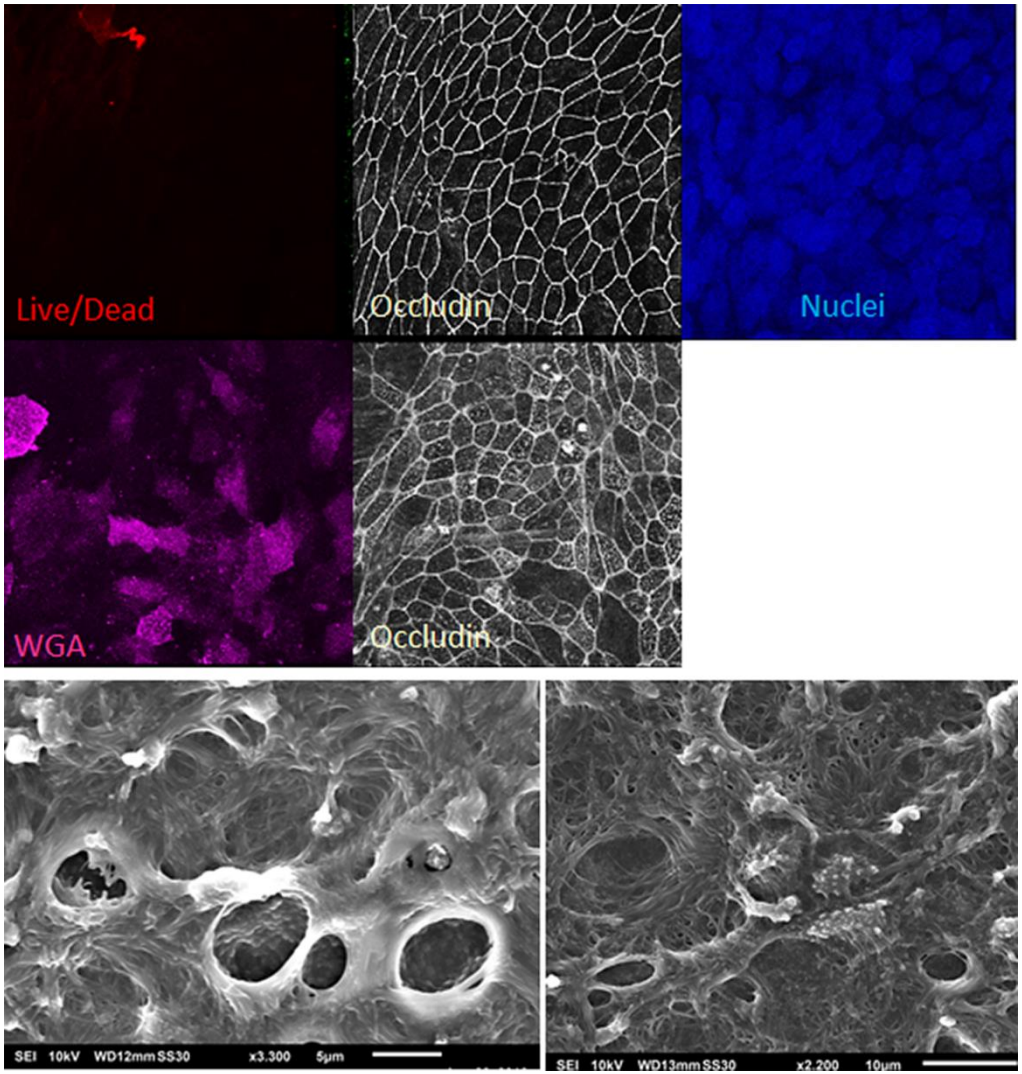
Supplementary Figure S1a: CLSM and SEM analyses of SAE cells grown under perfusion. The upper and middle panels depict CLSM analyses of SAE cells grown for 7 days under perfusion. Well-developed tight junctions (occludin, white) and cilia formation (lilac, middle panel) are observable as well as high rates of live cells as illustrated by using the Live/Dead Cell Viability Staining (Thermo-Fisher Scientific) (red, upper panel). Nuclei were stained using Draq5 (blue). The lower panel shows SEM of intact SAE epithelia. ***Supplementary Figure S1b: Live cell imaging of SAE cilia movement upon stimulation with beads (snapshot).*** Cilia were stained using WGA (blue), latex beads are depicted in red.

Supplementary Figure S2: Different A. fumigatus (ATCC46645) morphotypes (conidia, swollen conidia, germlings and hyphae) are depicted.

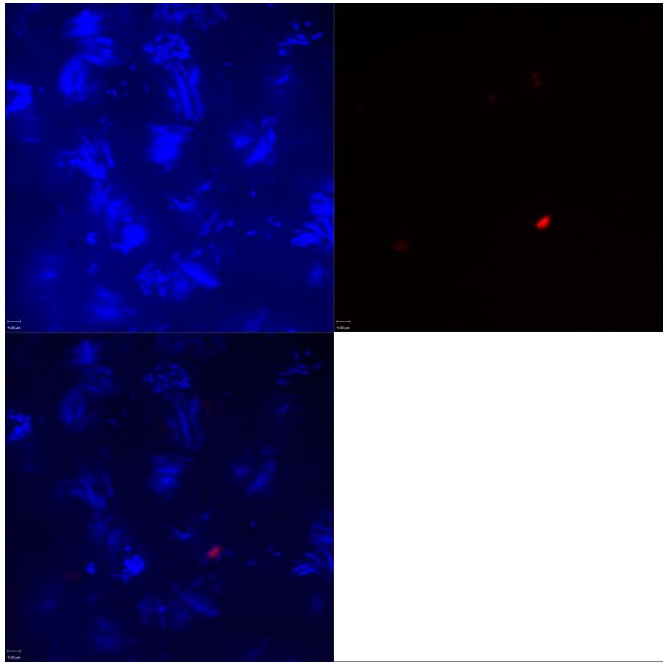
Supplementary Figure S3: CLSM analyses revealed apical DC aggregation around ia hyphae. To see if PAMPs are sufficient for DC attraction, apical epithelial layers were exposed to ia hyphae (red) for 48h. CFSE-DCs (green) were attracted to high numbers by ia hyphae (overlay). In addition a live/dead staining was performed (blue), which illustrated the killing of the applied fungi.

Supplementary Figure S4: dsRed Aspergillus fumigatus hyphae are illustrated by light (left) and fluorescence (right) microscopy.

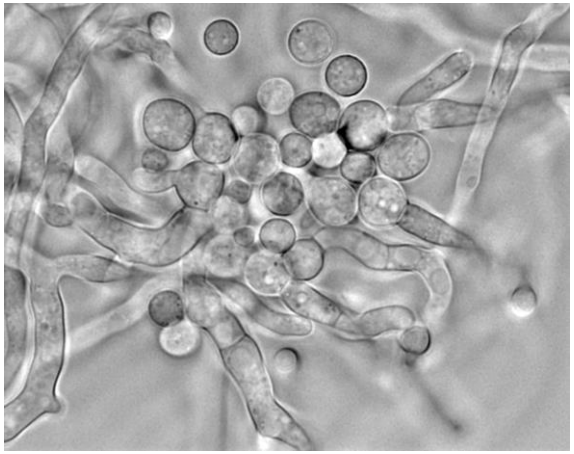
Supplementary Figure S1a



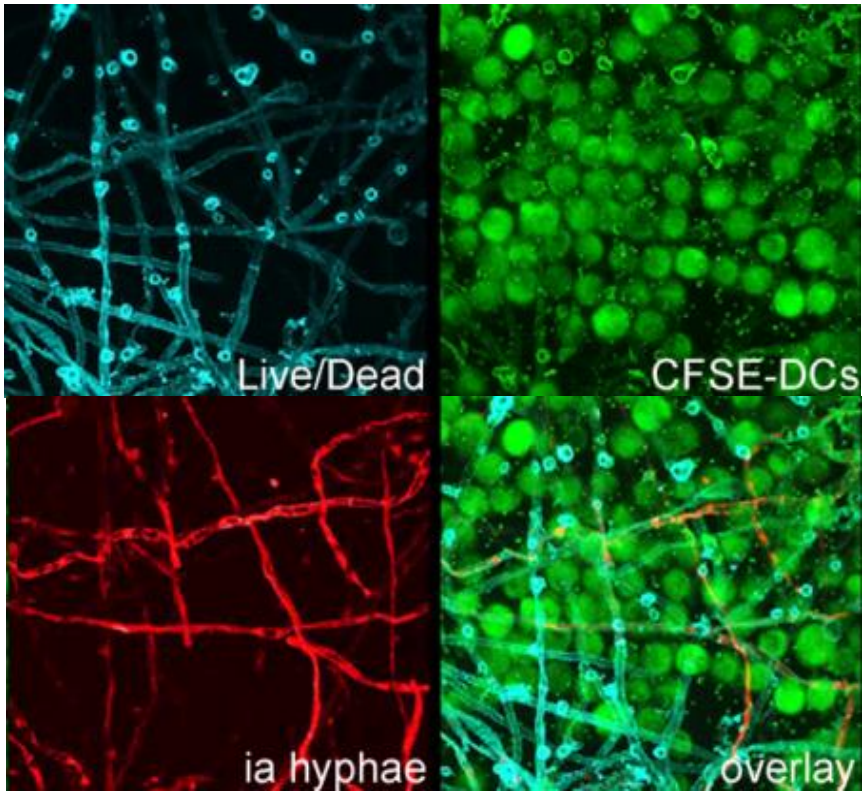
S1b



Supplementary Figure S2



Supplementary Figure S3



Supplementary Figure S4