# "Invasive aspergillosis on-a-chip" – a novel disease model to study *Aspergillus fumigatus* infection in the human lung

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## Introduction:

Invasive fungal infections in immunocompromised patients are drastically increasing. *Aspergillus fumigatus,* an ubiquitous airborne pathogen, is one of the most common fungal pathogens. The infection occurs primarily in the lung, where fungal conidia germinate and grow into filamentous bodies (hyphae), penetrate the epithelium and invade into the bloodstream, where they can disseminate into various organs. Despite new methods improving our comprehension of pathological mechanisms, human cell-based models to investigate invasive aspergillosis are rare and include either a limited number of involved cell types or insufficient mimicry of the human alveolar structures. Here, we established an "invasive aspergillosis on-a-chip" model to investigate *A. fumigatus* infection by conidia in various clinical situations. Based on this novel disease model, the advanced automated image analysis of 3-dimensional confocal microscopy data allowed us to visualise and quantify numerous parameters of hyphal growth, including length and branching levels.

## The lung-on-a-chip

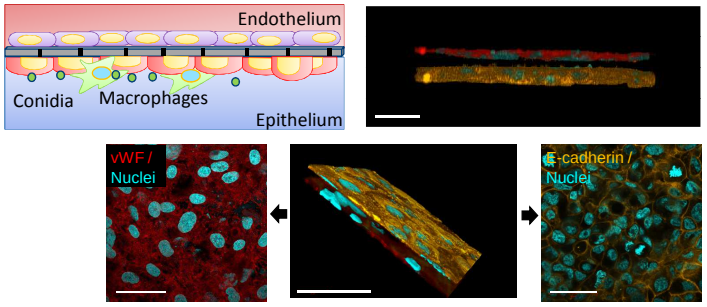


**Figure 1:** The lung-on-a-chip included a porous membrane, an upper (blood) chamber comprising human endothelial cells (stained with von Willebrand factor, red), and a lower (lung) chamber including epithelial cells which expressed tight-junction protein E-cadherin (yellow). Scale bars: 50 µm

## The work flow

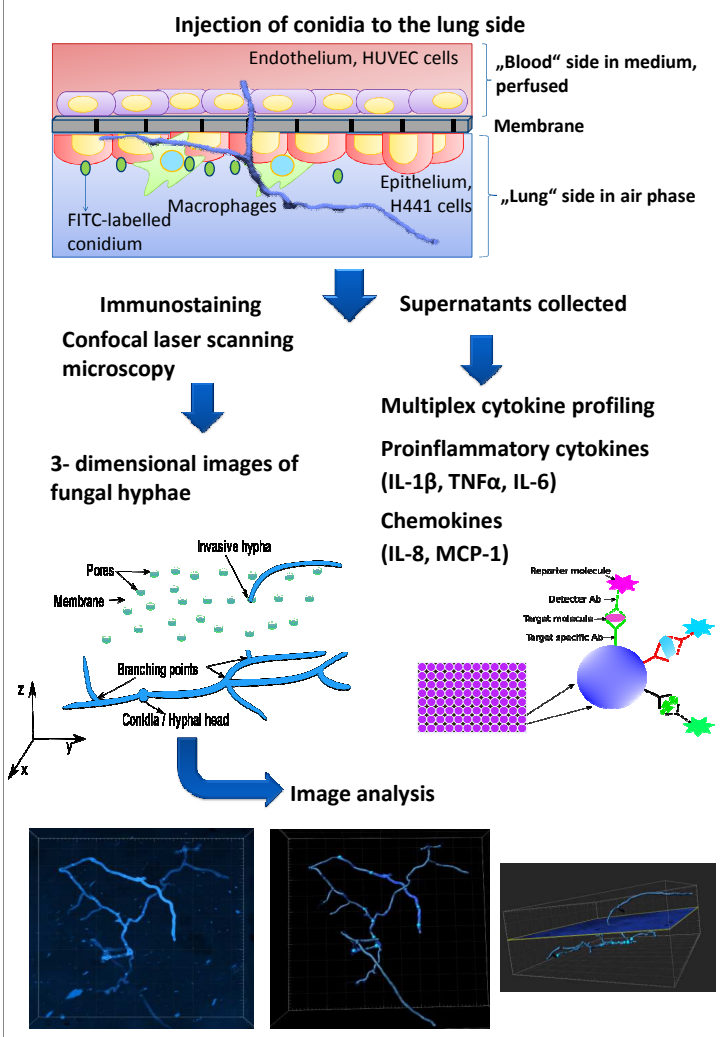


**Figure 2:** The work flow: from the injection of conidia into the lung side to the sampling methods (immunostaining and cytokine profiling). The z-stack images of fungal hyphae were taken using a confocal laser scanning microscope. Quantification of these 3D images was done by advanced automated image analysis (IMARIS® software).

## The effect of macrophages on the hyphal growth

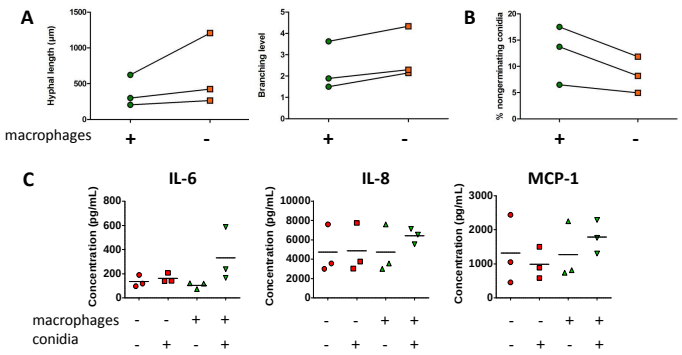


**Figure 3: (A)** Alveolar macrophages partially inhibited the hyphal growth, **(B)** reduced conidial germination and **(C)** induced production of proinflammatory cytokines and chemokines (n=3 independent experiments with 3 different macrophage donors, (A) and (B): mean of 6 hyphae per condition per experiment).

## Infiltrating leukocytes eradicated fungal growth

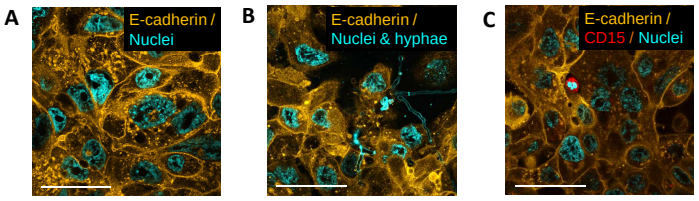


**Figure 4:** The perfusion of isolated healthy human leukocytes into the model, which already included macrophages, completely abrogated the hyphal growth in the lung side in this experiment. The isolated leukocytes were perfused into the blood side of the biochip, the conidia were subsequently added to the lung side where they interact with the infiltrating immune cells. **(A)** Control without fungal infection. **(B)** After 14 h of perfusion, hyphal growth and invasiveness were observed in the model without leukocyte perfusion, **(C)** while fungi were eradicated in the leukocytes perfused model. Scale bars: 50 µm

## Conclusion and outlook:

Our results showed that the addition of human macrophages to the biochip partially reduced the hyphal length and branching level and induced the production of proinflammatory cytokines and chemokines. The perfusion of isolated healthy human leukocytes reduced the fungal growth in the lung side. The development of this "invasive aspergillosis on-a-chip" model is very promising due to its potential contribution to the understanding of fungal pathogenicity in invasive aspergillosis. The model can be modified to mimic the physiological conditions in immunocompromised patients, whilst these chips may also provide reliable tool for animal-free drug screening.

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References: (1) A microfluidically perfused three dimensional human liver model. Rennert et al., Biomaterials, 2015; (2) Monocyte-induced recovery of inflammation-associated hepatocellular dysfunction in a biochip-based human liver model. Gröger et al., Scientific Reports, 2015