Modeling of intravenous caspofungin administration using an intestine-on-chip reveals altered *Candida albicans* microcolonies and pathogenicity

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The human intestine is a major reservoir of the normally commensal fungus *Candida albicans* and the main source for life-threatening systemic candidiasis[1,2]. To dissect the fungal-host interactions, conventional 2D *in vitro* models are commonly used. However, these models lack biological complexity and are less suitable for evaluating antifungal drug administration routes and the drug concentration gradients across multilayered tissue barriers as observed *in vivo*.

Therefore, a 3D intestine-on-chip model[3] was leveraged to investigate fungal-host interactions and evaluate the antifungal activity of caspofungin. This model includes a vascular compartment with endothelial and immune cells, an intestinal compartment with villus- and crypt-like structures, and peristaltic flow in both compartments. The model was infected with either a wild-type (SC5314) or caspofungin-resistant (110.12) strain of *C. albicans*. Caspofungin was vascularly perfused to simulate the intravenous treatment of candidemia. Complementary to biomolecular analyses, we developed an automated image analysis pipeline that provides 3D morphological characterization of fungal objects. Additionally, this pipeline provides quantification of the epithelial tissue architecture, the interaction between fungal objects and tissue structures, as well as the level of fungal invasion and translocation. We demonstrated that *C. albicans* invades the epithelial tissue and translocates to the vascular compartment in the model. Moreover, *C. albicans* induced severe host tissue damage by disrupting apical cell-cell contacts, increasing intestinal permeability, and promoting vascular

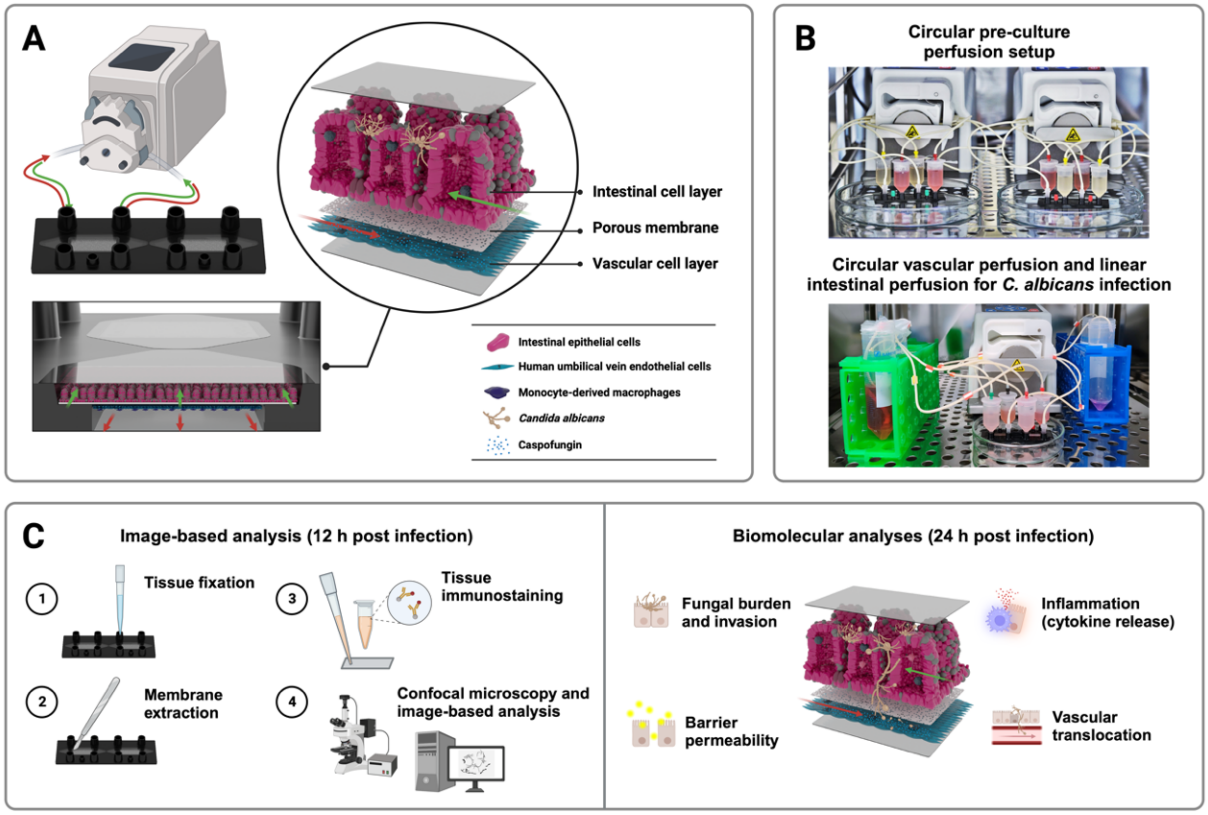

Figure 1: Schematic illustration of the intestine-on-chip candidiasis model with subsequent image-based and microbiological analyses for testing the antifungal activity of caspofungin.

inflammation. Subsequent caspofungin treatment restored vascular and intestinal host tissue integrity. Further, fungal burden, tissue invasion, and vascular translocation were concentration-dependently reduced and accompanied by decreased inflammation. Advanced image-based analysis revealed a reduction in fungal biomass associated with the formation of more compact microcolonies and a diminished surface-to-volume ratio, which indicated a decreased exposure to the epithelial tissue after caspofungin treatment. Contrary to the wild-type strain, caspofungin showed limited effects even at high therapeutic concentrations in the case of infection with the caspofungin-resistant clinical isolate.

Collectively, the intestinal candidiasis-on-chip-model can be leveraged for in-depth *in situ* characterization of antifungal treatment efficacy, emerging fungal drug resistances, and associated changes in host-pathogen interactions.

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