

Image-based analysis of *Candida albicans* infection in a gut-on-chip model

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Candida albicans: commensal and pathogen

In vitro

- High throughput and dissecting molecular mechanisms
- Simple, easy, and cheap
- Not always recapitulate physiology

Systemic disease

↑

Pathogen

↑

Commensal

In vivo

- Complex environment, holistic, systemic outcomes
- Ethical and practical limitations
- Interspecies differences

Gut-on-chip experimental model

Epithelial compartment

Membrane

Vascular compartment

Cell medium

- Multicellularity
- Immune cells
- Tissue architecture
- Compartmentalization
- Dynamic system
- Human cells
- Imaging

Near-physiological

- Endothelium
- Macrophages from human PBMCs
- Intestinal cells

Data generation

500 μm

C. albicans
Nuclei
F-actin

- Extraction of the membrane
- Staining
 - *C. albicans*
 - Nuclei
 - F-actin
- Confocal microscopy

Image analysis pipeline

Morphometrics of microcolonies

(a) Original signal (b) Segmented image (c) Sub-region

(d) Sub-region local enhancement and segmentation (e) Reconstructed image

$$\text{Compactness} = \frac{V_{\text{object}}}{V_{\text{fitting ellipsoid}}}$$

$$\text{Flatness} = \frac{\text{second axis of ellipsoid}}{\text{third axis of ellipsoid}}$$

Quantification of tissue architecture

Membrane
Cell surface

Tissue thickness

Spatial host-fungus interaction

$$V_{\text{comp}} = \frac{V_{i\text{comp}}}{V_i}$$

comp: one of the three compartments
 $V_{i\text{comp}}$: the proportional overlapping volume of microcolony i with comp
 V_i : overall volume of microcolony i

- Quantification of epithelial tissue penetration and vascular invasion
- Localization of microcolonies on epithelial tissue landscape

Application I: Caspofungin effect on *C. albicans* infection in a gut-on-chip model

Experimental setup

Caspofungin at patient-relevant concentrations

SC5314

110.12: clinical isolate, resistant to echinocandins

C. albicans Nuclei

Control

1 μg/ml Caspofungin

0.25 μg/ml Caspofungin

4 μg/ml Caspofungin

Control

4 μg/ml Caspofungin

1 μg/ml Caspofungin

6 μg/ml Caspofungin

SC5314, 12 hpi

110.12 (echinocandin-resistant), 12 hpi

Wild type SC5314

Clinical isolate 110.12

Wild type SC5314

Clinical isolate 110.12

Wild type SC5314

Clinical isolate 110.12

Application II: Quantitative assessment of the preventive effect of butyrate in an inflammatory-bowel-disease-on-chip model

Inflammatory bowel disease (IBD)

- Chronic inflammation of the gastrointestinal (GI) tract
- Severe and rising healthcare issue in the world, especially in industrialized countries

Experimental setup

Gut-on-chip has been developed to a disease-model mimicking IBD using dextran sodium sulfat (DSS)

- ✓ Reduced expression of E-Cadherin
- ✓ Elevated levels of proinflammatory cytokines such as IL-6 and IL-8

Control

But

DSS

But+DSS

Condition

Condition

Condition

Condition

- ✓ Preventive effect of butyrate on the tissue damage caused by DSS
- ✓ Increased flatness in microcolonies under DSS
- ✓ Reduced fungal volume and invasion after pretreatment with butyrate

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