Development of an improved in vitro model of human intestinal follicle associated epithelia to study cellular and molecular interactions of Candida albicans with M cells

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Development of an improved *in vitro* model of human intestinal follicle associated epithelia to study cellular and molecular interactions of *Candida albicans* with M cells.

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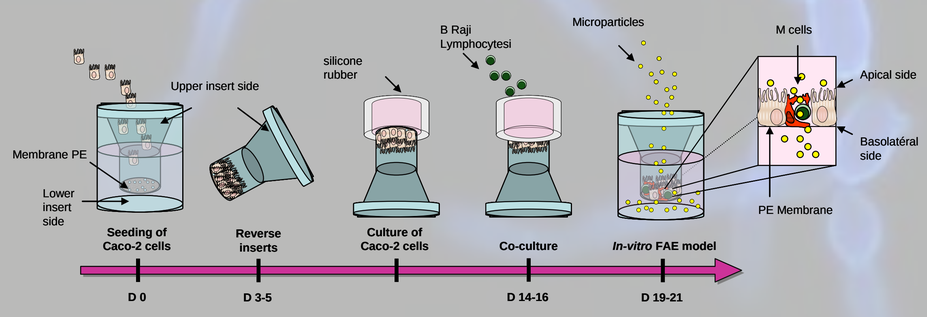
ABSTRACT

Candida albicans is a commensal inhabitant of the human mucosa causing harmful invasive infections in immuno-compromised patients, taking origin mainly from the gastro-intestinal tract. A better understanding of the mechanisms by which *C. albicans* interacts with the intestinal mucosa will improve our knowledge of the physiopathology of disseminated candidiasis. *C. albicans* can grow upon mucosal surfaces in both the yeast and the hyphal forms, the transition from the yeast to the hyphal form playing a key role in its virulence. Mucosal immunity contributes to both commensalism and pathogenicity of the fungus, possibly through presentation of *C. albicans* antigens to the underlying organized lymphoid structures *via* transcytosis that could probably be mediated by the specialized epithelial M cells. With this aim, we developed an *in vitro* model of the human intestinal Follicle Associated Epithelium (FAE) where enterocytes of the Caco-2 cell line in close contact with mucosal lymphocytes differentiate in M cells.

Studying adherence, invasion and translocation of *C. albicans* across co-cultures suggest that *C. albicans* interacts differentially with M cells / enterocytes co-cultures as compared to mono-layers of Caco-2 cells alone. The uptake mechanism allowing *C. albicans* to translocate across the co-culture model is under investigation. Moreover, the respective contribution of the yeast and hyphal forms to this process will be studied using KO mutants of *C. albicans* unable to produce hyphae. Finally the cytokine production resulting from *C. albicans* and M cells / Caco-2 co-cultures interaction will be studied.

INVERSED FAE MODEL

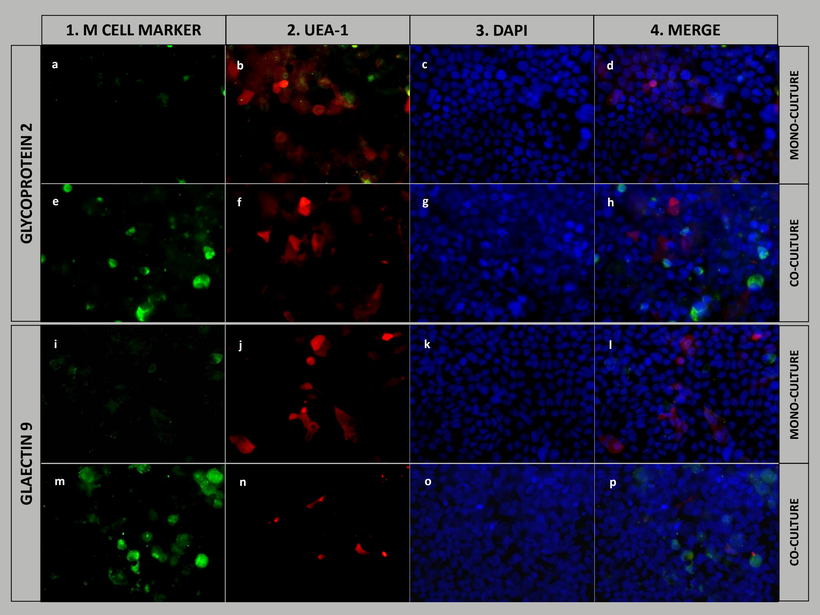
FIGURE 1. Time scale of the protocol to obtain an optimized inverted in vitro FAE model as described by Des Rieux et al. (1)



VALIDATION AND CHARACTERISATION OF THE FAE MODEL

Localisation of M cells in the co-cultures

FIGURE 2. Identification of M cells by immunofluorescence analysis.



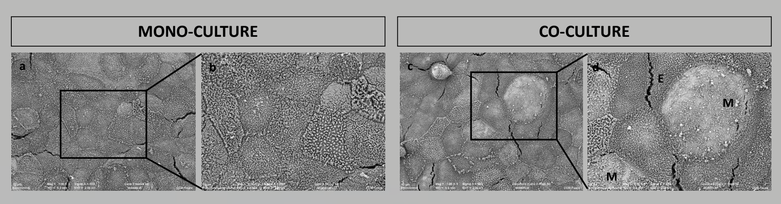
GP2 and Galectin-9 expression was increased on the apical surface of the co-cultures, suggesting the presence of M cells in the layer of cells (2)

The UEA staining decreases in the co-cultures layers as compared to mono-cultures conditions, suggesting changes in the apical membrane components induced by the contact of lymphocyte B with enterocytes (2).

Mono-cultures were used as a control.

Method:
1. M cells (green) on the cell layers were stained (l and m) with a polyclonal IgG goat anti-galectin 9 antibody (Santa cruz) or (a and e) with a polyclonal IgG rabbit anti-GP2 (Imgenex) and were revealed with an Alexa fluor 488 rabbit anti-IgG goat (Invitrogen) or an Alexa fluor 488 goat anti-IgG rabbit antibody respectively (Invitrogen).
2. Enterocytes (red) were labelled with the UEA-1 (*ulex europaeus agglutinin 1*) lectin ((Sigma).
3. Nuclei (blue) were revealed with a DAPI staining (Invitrogen).
4. Merge: Overlap of the three different staining.

FIGURE 3. Visualization of M cells by Scanning Electron Microscopy (SEM) analysis.



Mono-cultures: <1% of M cells
Co-Cultures: 5% of M cells

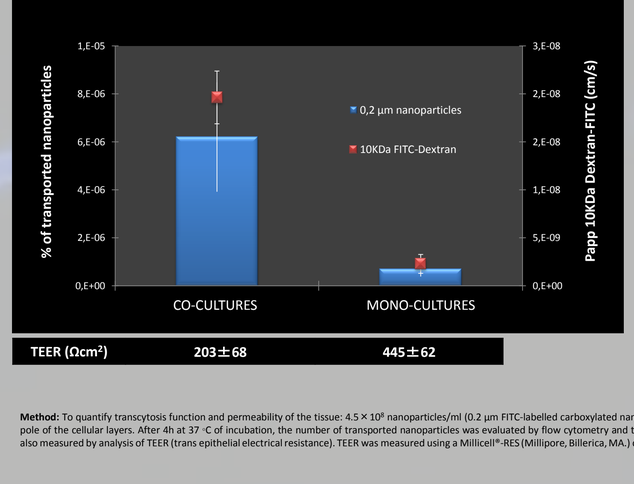
Method: Cell layers were fixed and processed for SEM analysis. M cells were identified by their lack or fewer microvilli at their apical surface (c and d). Mono-cultures were used as control (a and b) (M = M cell ; E = enterocyte).

References

- **des Rieux et *al*., 2007,** European journal of pharmaceutical sciences, 3 0 (2 0 0 7), 380–391
- **Pielage et *al*., 2007,** The International Journal of Biochemistry & Cell Biology, 39 (2007), 1886–1901

Assessment of functionality and integrity of the model

FIGURE 4. Assessment of functionality and integrity of the FAE model.



TEER (Ωcm^2)	203±68	445±62

Transepithelial electrical resistance (TEER) decrease is considered to be a result of Caco-2 cell conversion into M cells. The observed TEER values were in the range reported in the literature for both co-cultures and mono-cultures with good tightness (1).

10KDa FITC-Dextran apparent permeability (*P*app) were 10 fold higher in co-cultures as compared to mono-cultures, suggesting that this molecule may cross the epithelial barrier at the paracellular level and/or by transcytosis (1).

The number of transported nanoparticles was 20 fold higher in co-cultures than in mono-cultures suggesting that this molecule may cross the epithelial barrier by transcytosis (1).

Method: To quantify transcytosis function and permeability of the tissue: 4.5×10^8 nanoparticles/ml (0.2 µm FITC-labelled carboxylated nanoparticles)(Invitrogen) and 1mg/ml of 10KDa FITC-dextran suspended in HBSS, were added to the apical pole of the cellular layers. After 4h at 37 °C of incubation, the number of transported nanoparticles was evaluated by flow cytometry and the amount of transported 10KDa FITC-dextran was measured by fluorimetry. Integrity of the tissue was also measured by analysis of TEER (trans epithelial electrical resistance). TEER was measured using a Millicell®-RES (Millipore, Billerica, MA.) ohmmeter. Values appear down each group results. Each condition was tested in triplicate.

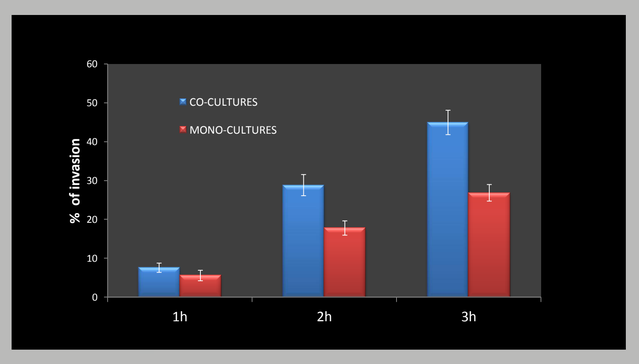
INTERACTION OF *C. albicans* WITH FAE MODEL

FIGURE 5. Adherence of* C. albicans *SC 5314

Adherence of *C. albicans* SC 5314 was 2 fold higher in co-cultures as compared to mono-cultures, suggesting that *C. albicans* interacts preferentially with M cells of the co-culture layers.

Method: The reference strain *C. albicans* SC 5314 was grown in liquid YPD (1% yeast extract, 2% bacto-peptone, 2% D-glucose) medium at 37° C overnight, with shaking. Prior to use *C. albicans* cells were diluted to a OD of 0,3 (600nm) into fresh liquid YPD medium and grown to log phase for 2 h at 37° C. Cultures of epithelial cells were infected with 4 x 10^4/ml of *C. albicans* log phase, for 30 minutes. After rinsing, cultures were fixed and stained with a rabbit polyclonal anti-*C. albicans* antibody (Acris) and with an Alexa fluor 488 goat anti-rabbit secondary antibody (Invitrogen). The percentage of adherence was determined as the ratio of the number of adherent yeasts to the number of *C. albicans* cells inoculated. Each condition was tested in triplicate.

FIGURE 6. Percentage of invasion of* C. albicans *SC 5314



Invasion of *C. albicans* was significantly increased in co-cultures after 2 hours of incubation as compared to mono-cultures, suggesting that *C. albicans* can penetrate more easily into layers containing M cells.

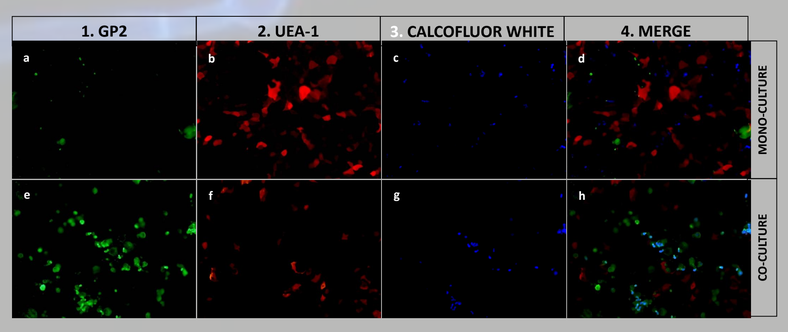
Method: *C. albicans* was grown as described above. Cultures were infected with 4 x 10^4/ml of *C. albicans* long phase, for 1, 2 or 3 hours. After rinsing, cultures were fixed and stained as described above. After permeabilisation both invasive and adherent yeasts were stained with calcofluor white. The percentage of invasive *C. albicans* cells was determined as the ratio of the number of [partially] internalized cells to the total number of interacting cells. Each condition was tested in quadruplicate

FIGURE 7. Determination of the* C. albicans *SC 5314 transport through the mono- and co- cultures.

After 24h of infection, translocation in co-cultures was 19 fold higher than in the mono-cultures, suggesting that translocation of *C. albicans* is facilitated by M cells.

Method: Cultures were infected with 10^7/ml of *C. albicans* log phase for 24 hours. Then, the totality of the basolateral medium was taken and sowed in YPD plates, at 30° C for 2 days. The number of colonies grown on plates was then counted. Finally, the percentage of translocation was estimated by the ratio of the number of colonies to the total number of yeasts inoculated. Each condition was tested in triplicate.

FIGURE 8. Co-localisation of* C. albicans *SC 5314 with M cells of the FAE model



Adherence of *C. albicans* was increased in co-culture conditions

Adherent yeasts mostly co-localised with GP2 stained cells, suggesting that yeasts preferentially adhered to M cells.

Method: Cultures were infected with 10^7 *C. albicans* log phase for 2 hours. After rinsing, cultures were fixed and stained as described above.
1. M cells (green) and **2. Enterocytes (red)** were labelled as describe above.
3. Adherent yeasts (blue) were revealed with a calcofluor white staining.
4. Merge: overlap of the three different staining.

CONCLUSIONS

Our results reported that adherence of *Candida albicans* SC5314 is greater in cultures containing M cells-like (co-cultures) than layers containing Caco-2 cells only (mono-cultures).

***Candida albicans* seems to adhere preferentially to cells expressing the Glycoprotein 2 (M cell label) on its apical side.**

***Candida albicans* can cross more easily through the cellular layers containing M cells-like as compared to Caco-2 cells alone.**

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