Characterization of the mucus lining a co-culture model of Caco-2/HT29-MTX cells

Yann Le Gouar, Nathalie Daniel, Thierry Pécot, Martine Morzel

Characterization of the mucus lining a co-culture model of Caco-2/HT29-MTX cells

Yann Le Gouar [a], Nathalie Daniel [a], Thierry Pécot [b] and Martine Morzel [a]

[a] STLO, INRAE, Institut Agro, Rennes, France [b] FAIIA core facility, Biosit (UAR 3480 CNRS - US 18 Inserm), Rennes University, France

Introduction

Context

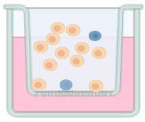
- Co-cultures integrating enterocyte-like cells (Caco-2) and goblet cells (HT29-MTX), are promising models of a functional epithelial barrier [1].
- It is expected that such cultures are lined by a mucus layer but the spatial characteristics of this layer is poorly described.

Objectives

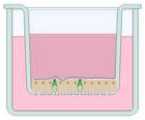
- To visualize mucins in a co-culture of Caco-2/HT29-MTX
- To set up a method of mucus characterization based on image analysis

Materials and methods

Caco2 + HT29-MTX (9:1) seeding density 2.10^5 cell/cm²



Incubation at 5% CO2 / 37°C 21 days



Differenciated monolayer: fixation with 4% PFA

Staining:
F-actin with phalloidin-rhodamine → **cytoskeleton**
Sialic acid with WGA-Alexa 488 → **mucins**

Confocal microscopy Zeiss-LSM 880

Photograph: Jordane Ossemond (STLO)

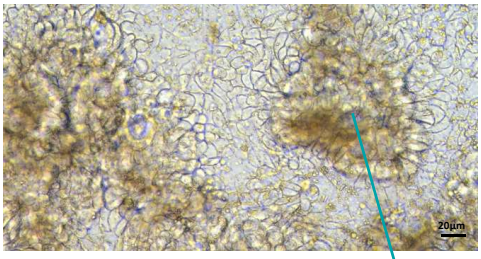
Ten 3-D images (80 stacked images on average) from 2 independent wells

Open-source software ilastik [2]

classification of pixels into three categories (corresponding to bright staining mucin clusters, diffuse mucin staining, background)

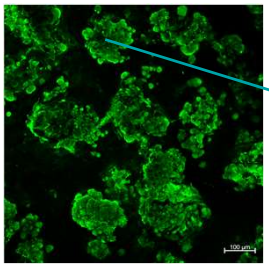
Results

1- Uneven distribution of the mucus



Observation of cultures by optical microscopy (X20)

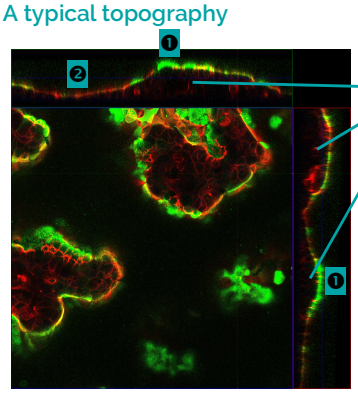
Cells are confluent and they form a monolyer but some cells are grouped and lined with a blurry material.



Observation of cultures after staining of sialic acid

Mucins are abundantly expressed as clusters

2- A typical topography



Observation of one stack of a 3-D reconstituted image

The culture is not planar. Domes (visible on z-views) are present. Height up to 135 µm.

Mucins are present mainly at the apical sides of domes (see ❶ bright staining: sites of secretion) but also between domes (see ❷ diffuse staining: secreted mucins spreading over the culture, loose mucus)

3- Development of an image analysis workflow to characterize the mucus structure

Machine learning: annotation of pixels representative of bright /diffuse staining on a subset of images, followed by automatic classification on all images.

Extraction of quantitative data

	Dense mucin clusters	Loose mucus
Average thickness	6.9 µm	21.2 µm
Average volume	183000 µm³	7502000 µm³

On-going: segmentation and classification of cells

Conclusions

- **Staining of sialic acid and confocal microscopy enable three-dimensional visualization of the mucus layer**
- **The mucus on the co-culture model is heterogenous in density**
- **The protocol of image analysis is suitable to extract quantitative data on the mucus layer (NB: lower thickness than *in vivo* data)**

These methodological developments open the way to studying the impact of digested food constituents or digested foods on the intestinal mucus

References:
(1) Béduneau et al. 2014. A Tunable Caco-2/HT29-MTX Co-Culture Model Mimicking Variable Permeabilities of the Human Intestine Obtained by an Original Seeding Procedure. Eur J Pharm Biopharm 87 (2), 290–298.
(2) Berg et al. 2019. A. Ilastik: Interactive Machine Learning for (Bio)Image Analysis. Nat Methods 16 (12), 1226–1232.