

# In vitro co-culture model of the inflamed intestinal mucosa

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Eva-Maria Collnot, [e.collnot@mx.uni-saarland.de](mailto:e.collnot@mx.uni-saarland.de)

Helmholtz Institute for Pharmaceutical Research Saarland

Department of Drug Delivery (DDEL)

Helmholtz-Institut für Pharmazeutische Forschung Saarland

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# Inflammatory bowel disease

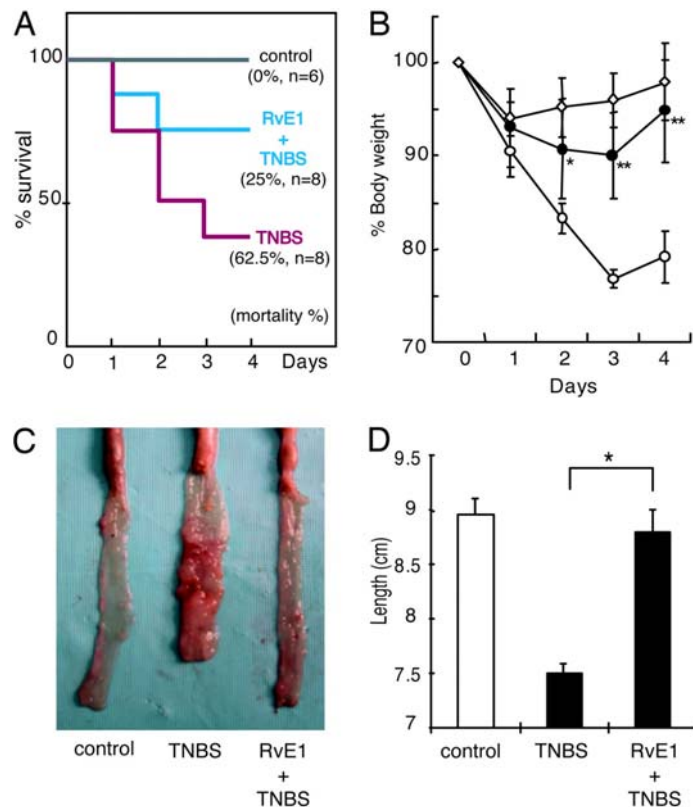
- A group chronic or recurrent inflammatory conditions of the colon and small intestine (Crohn's Disease and Ulcerative Colitis)
- Symptoms: diarrhea, weight loss, pain
- Treatment: induction and maintenance of remission using immunosuppressants, glucocorticoids, monoclonal antibodies (anti TNF- $\alpha$ )



# State of the art: animal models in drug/formulation development for IBD treatment

## Rodent colitis models

- Transgenic
- Chemically induced, e.g. TNBS, DSS



Symptoms:

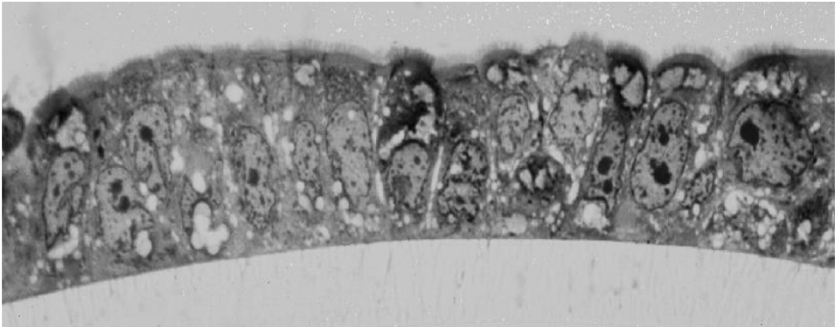
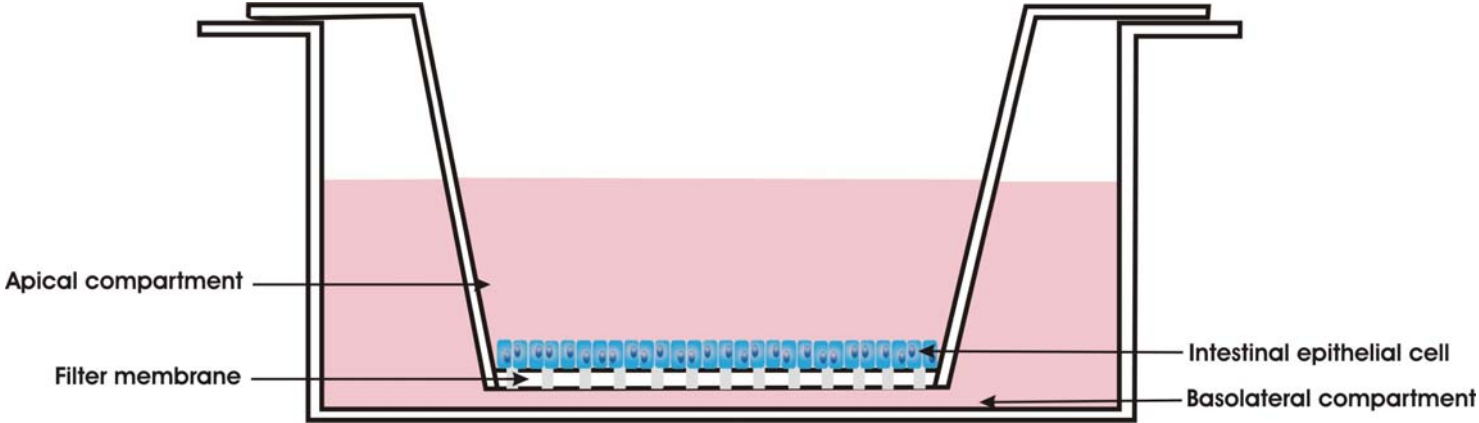
Diarrhea, rectal bleeding, weight loss, pain, colon perforation, sepsis, death

Evaluation of treatment: scoring system, histological stainings, weight and length of colon

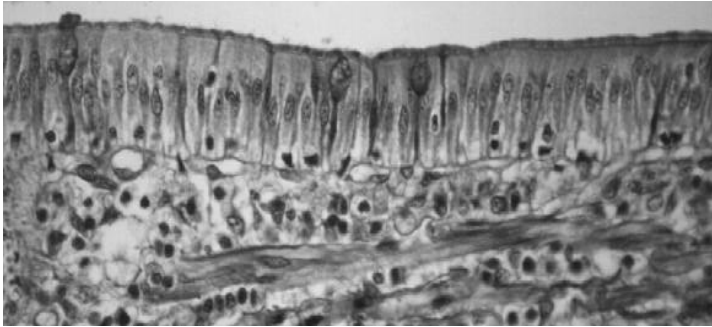
Issues: unethical, differences in species and pathogenesis

Arita M et al. PNAS 2005;102:7671-7676

# In vitro test systems for oral bioavailability

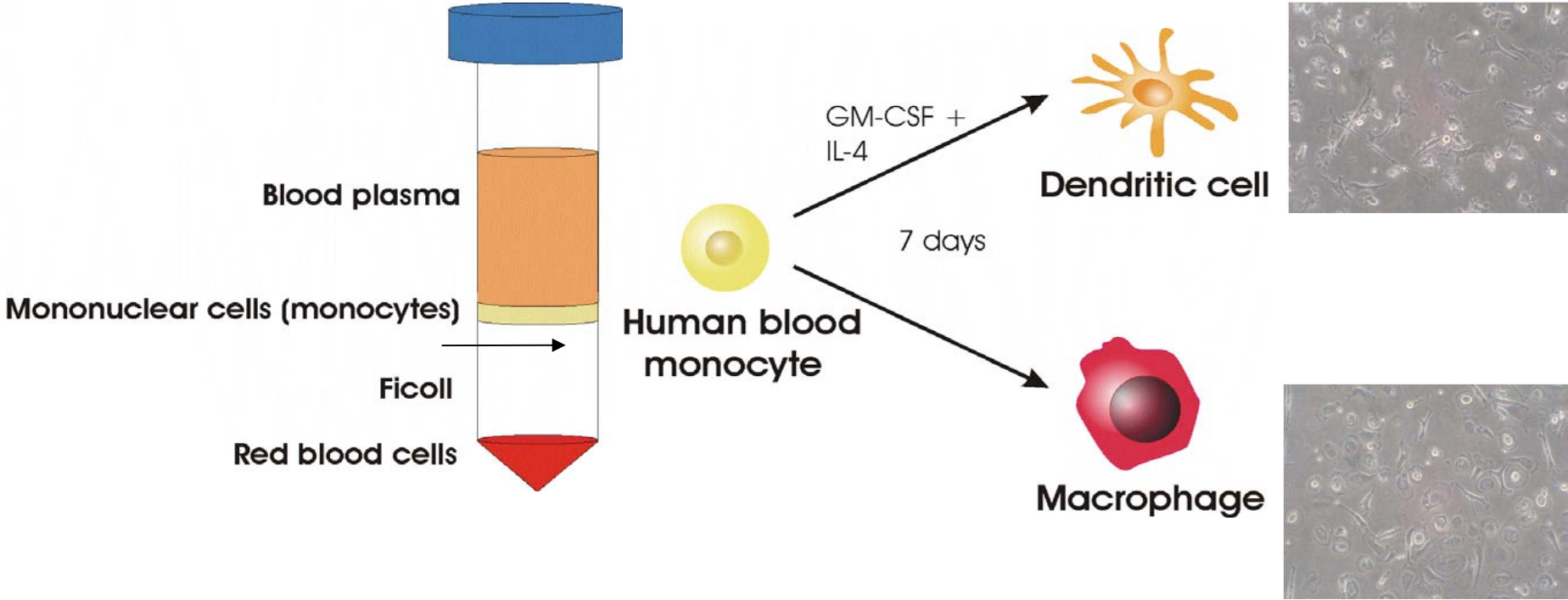


**Caco-2 monolayer**



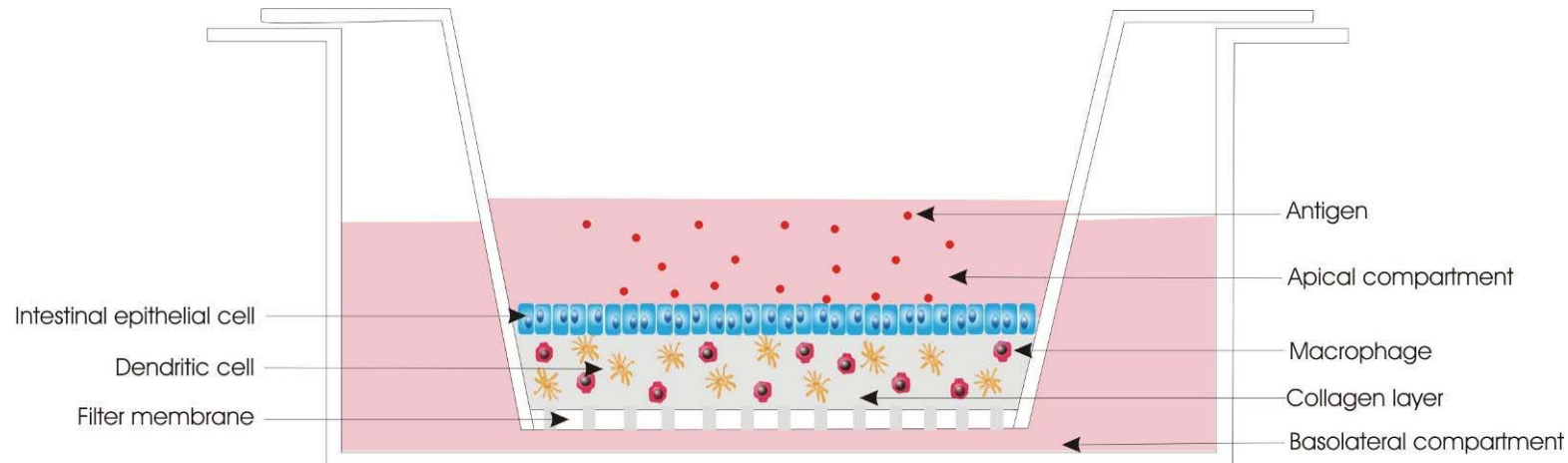
**Intestinal mucosa**

# Adding complexity: immune cells





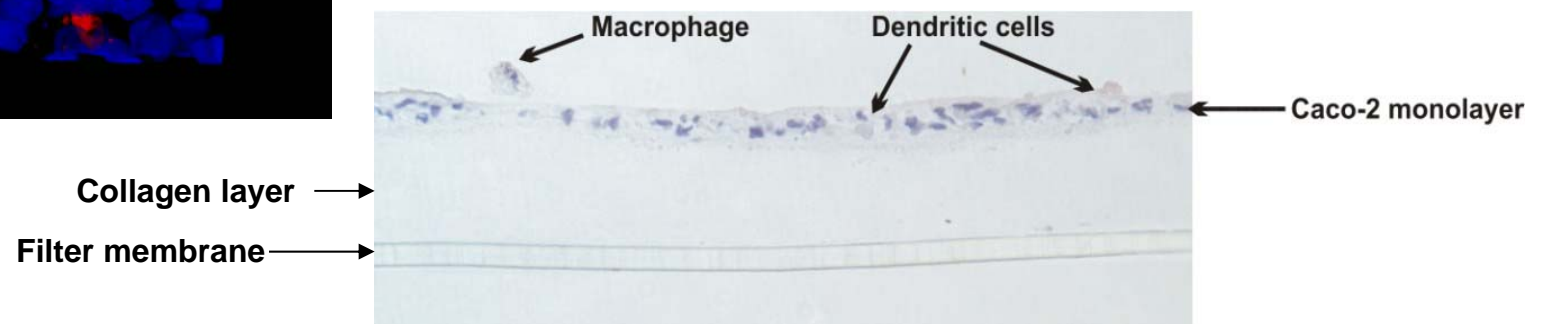
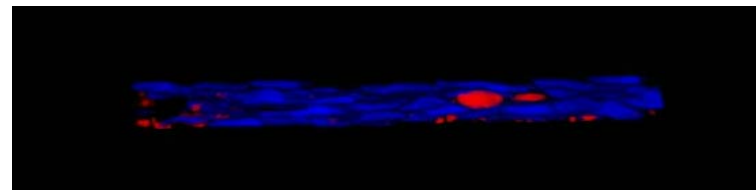
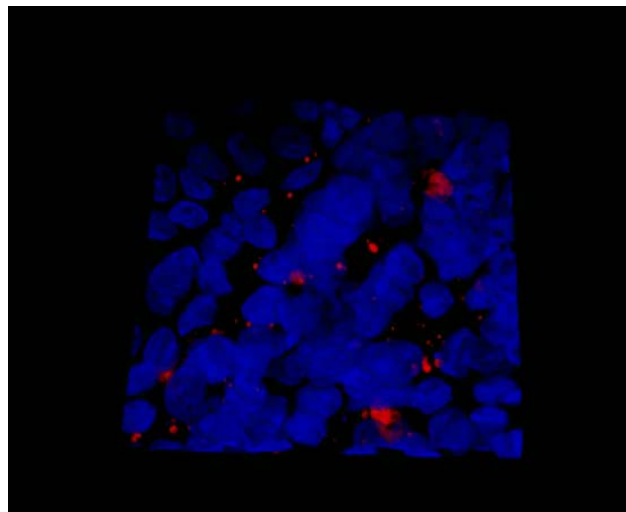
# 3D in vitro model of the inflamed intestinal mucosa



- Co-culture of Caco-2 intestinal epithelial cells with blood derived dendritic cells and macrophages
- Stimulation of inflammation by addition of lipopolysaccharides or pro-inflammatory cytokines (interleukin-1 $\beta$ ) to the cell culture medium
- Should reflect the relevant pathophysiological changes occurring in vivo: release of pro-inflammatory markers (IL-8, TNF- $\alpha$ ), re-organisation of tight junctional proteins, reduced barrier function, increased mucus production

# Pathophysiological changes in the 3D model

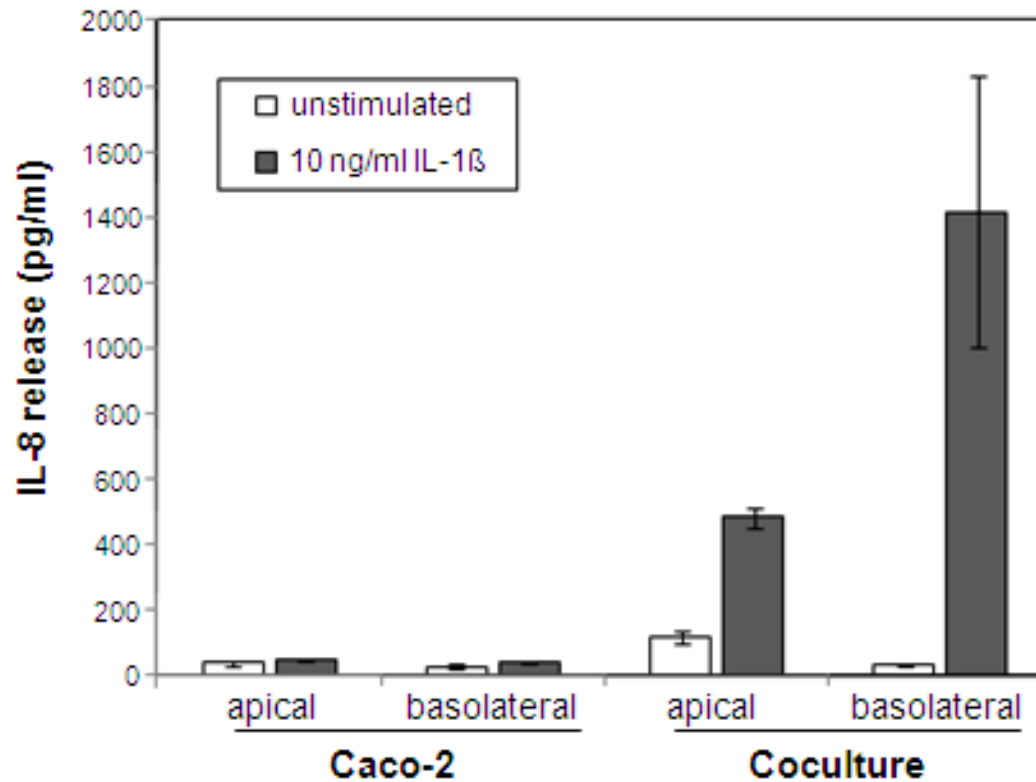
*Infiltration of immunocompetent cells  
(macrophages + dendritic cells)*



*Leonard et al, Mol Pharm: 7(6), 2103-19 (2010)*

## Pathophysiological changes in the 3D model

*Upregulation and release of pro-inflammatory markers, e.g. IL-8 or TNF- $\alpha$*

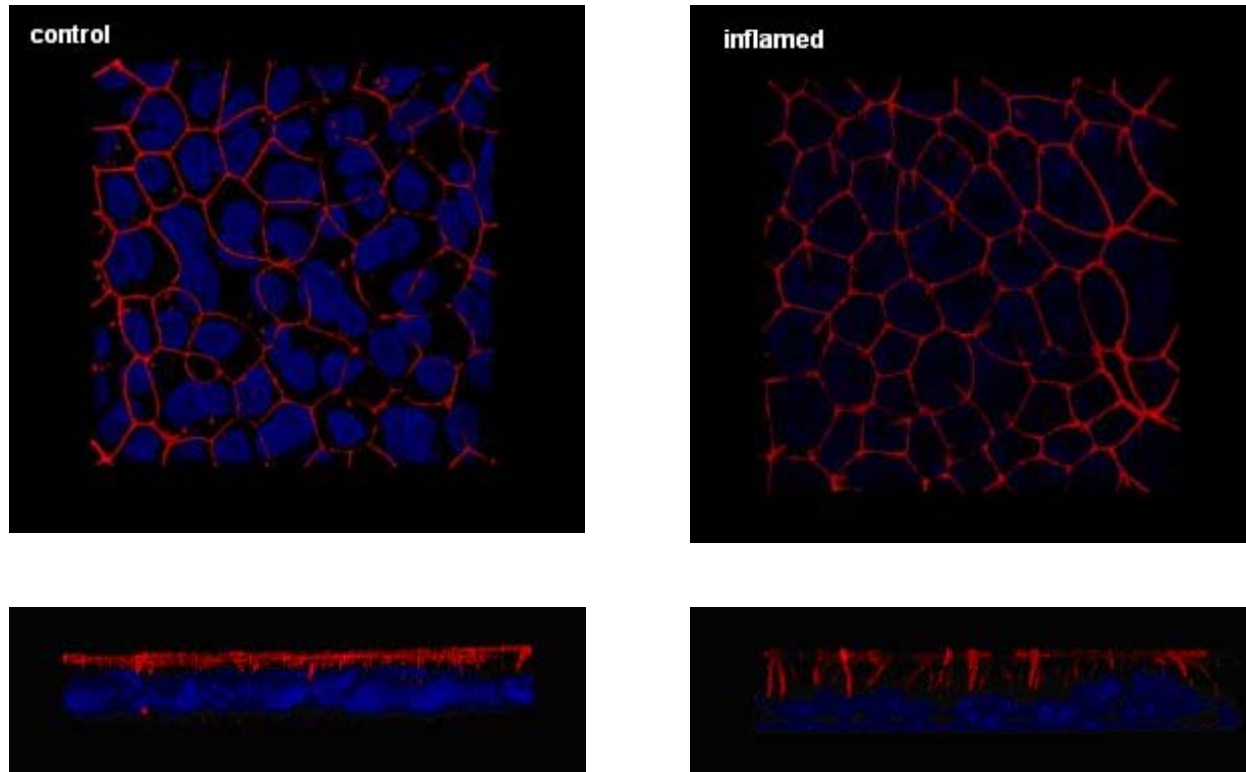


*Leonard et al, Mol Pharm: 7(6), 2103-19 (2010)*



# Pathophysiological changes in the 3D model

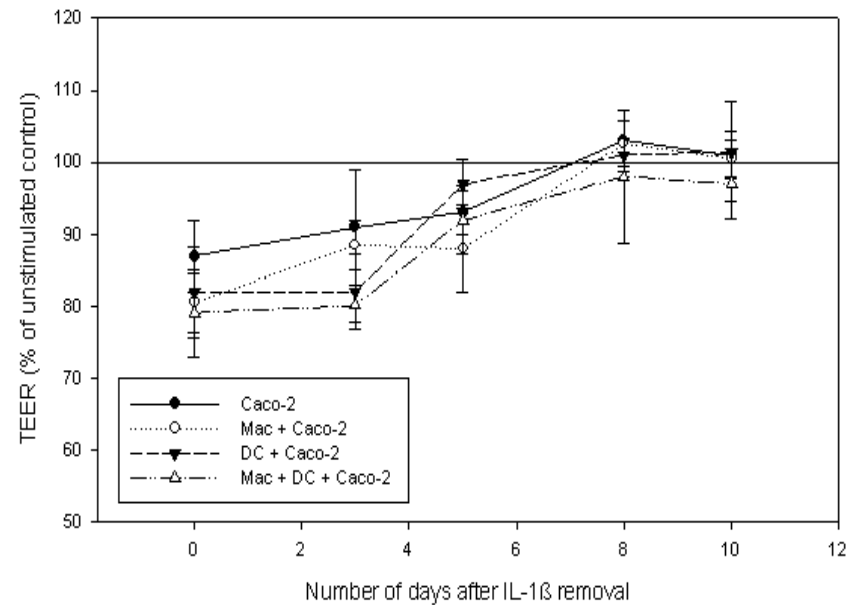
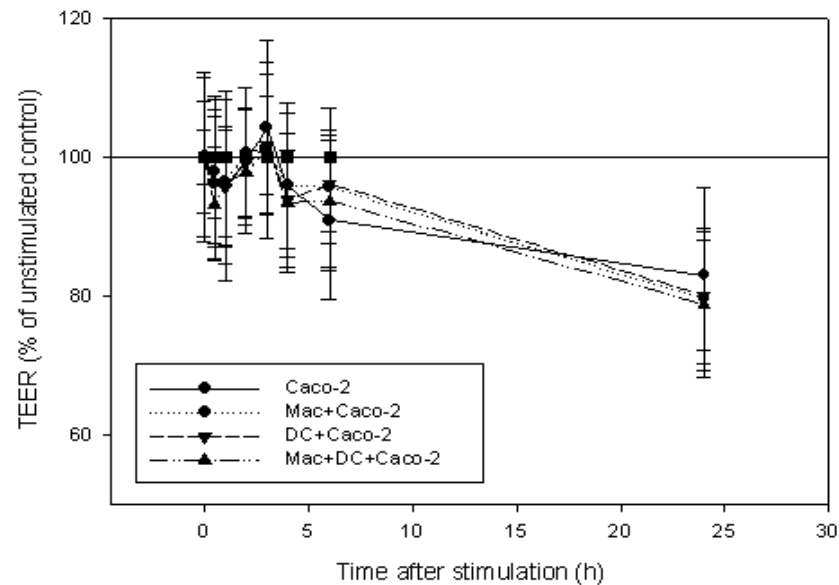
*Changes in tight junctional organization (ZO-1) ...*



*Leonard et al, Mol Pharm: 7(6), 2103-19 (2010)*

# Pathophysiological changes in the 3D model

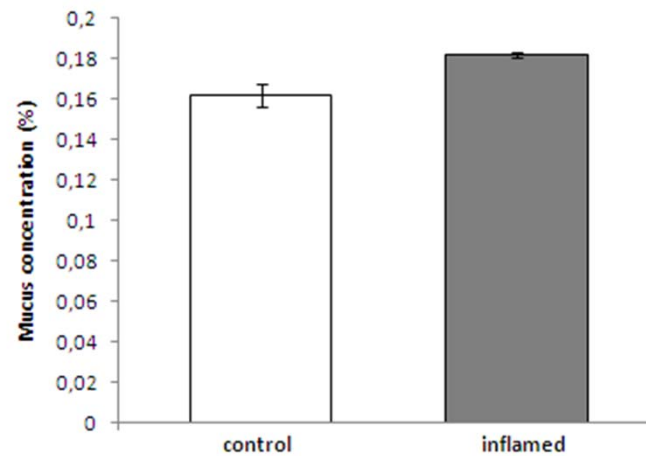
*... and barrier function*



Leonard et al, Mol Pharm: 7(6), 2103-19 (2010)

# Pathophysiological changes in the 3D model

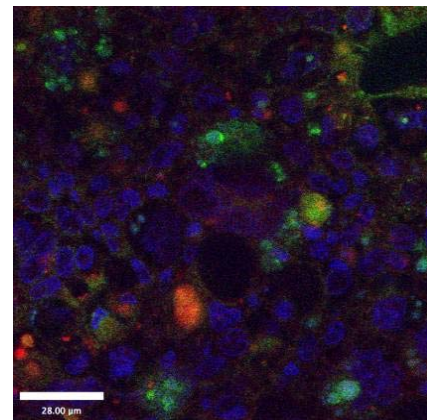
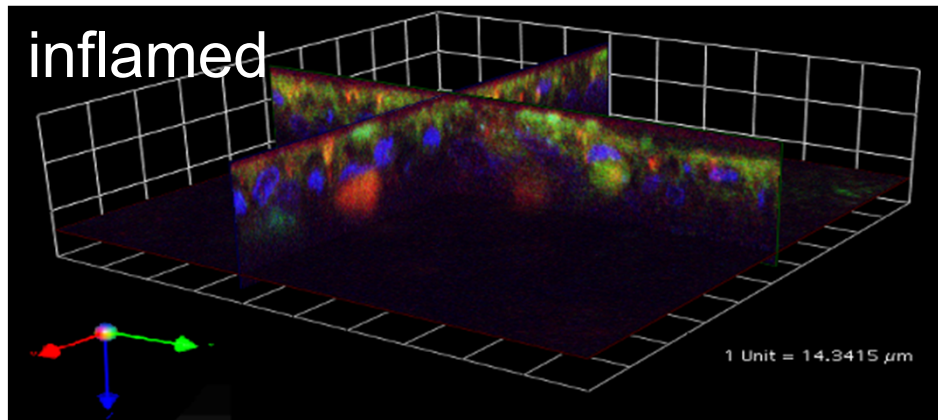
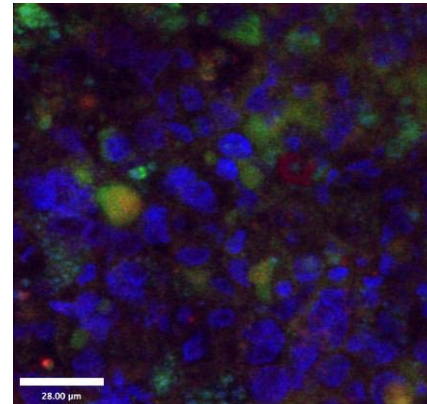
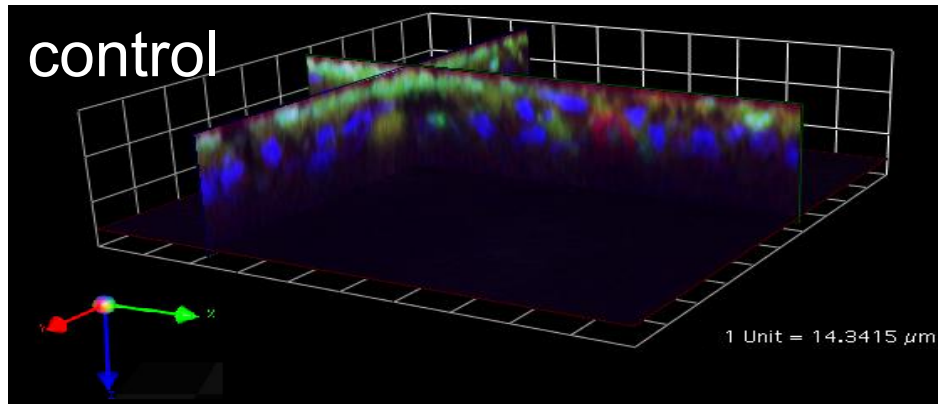
## *Increased mucus production*



*Leonard et al, Mol Pharm: 7(6), 2103-19 (2010)*

# Pathophysiological changes in the 3D model

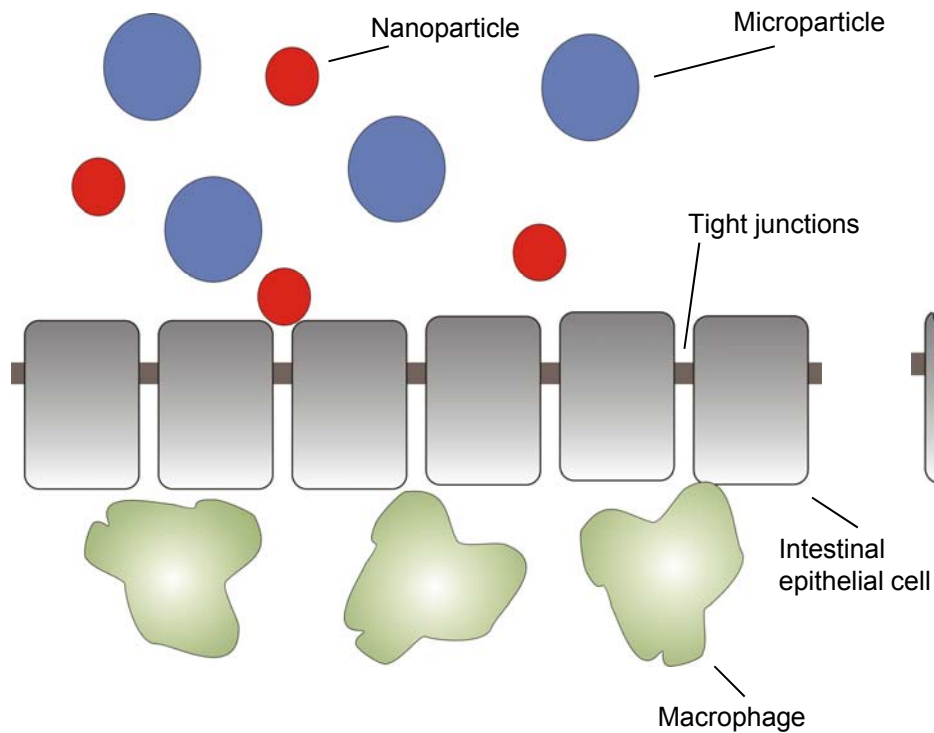
*Increased activity of immune cells*



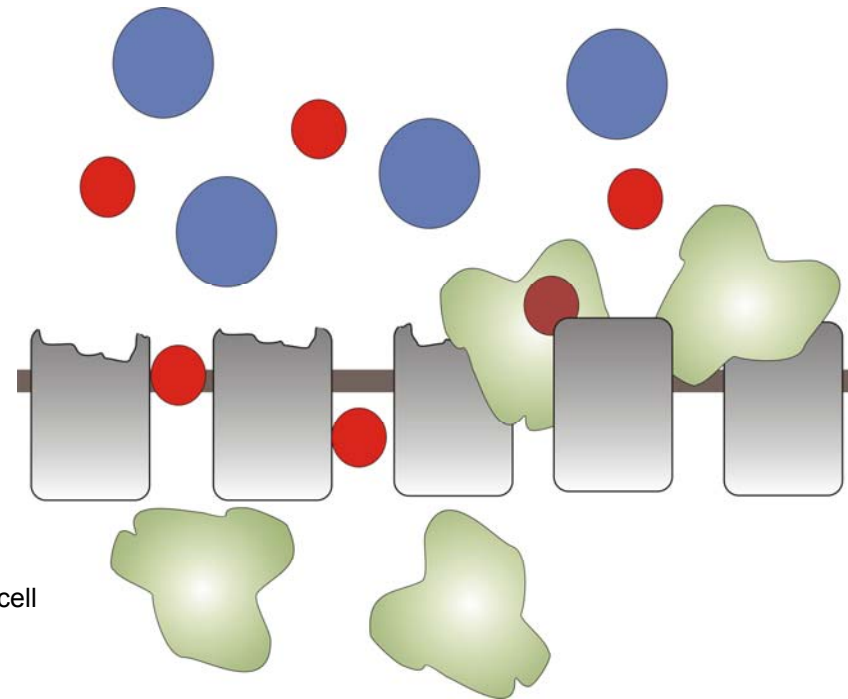
*Leonard et al, Mol Pharm: 7(6), 2103-19 (2010)*

# Pathophysiological changes in the inflamed mucosa: Threat or potential?

## Healthy mucosal barrier



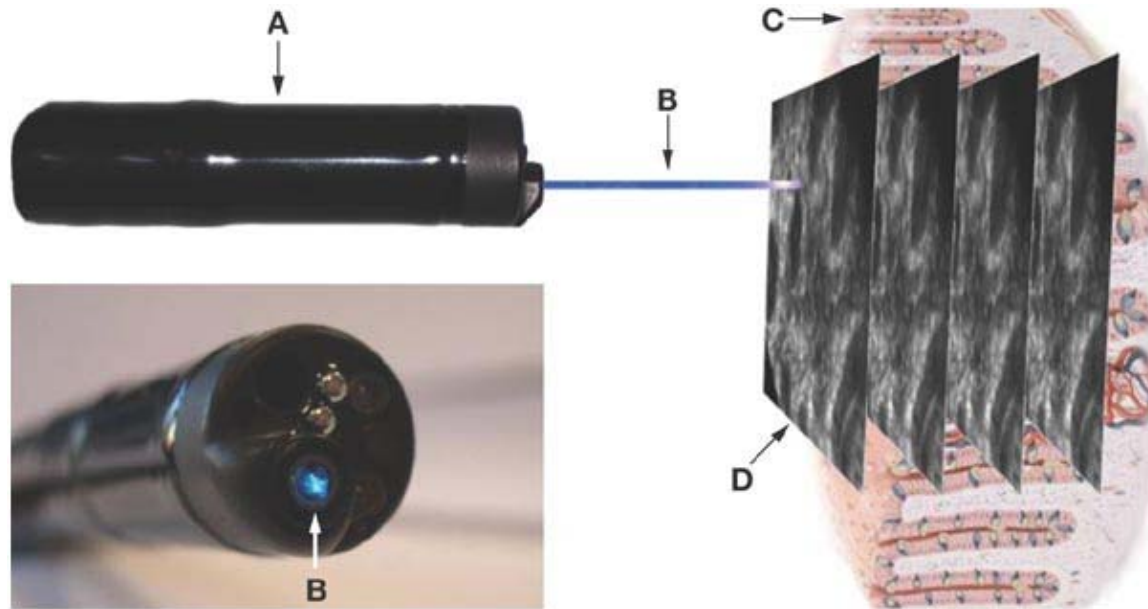
## Inflamed mucosal barrier



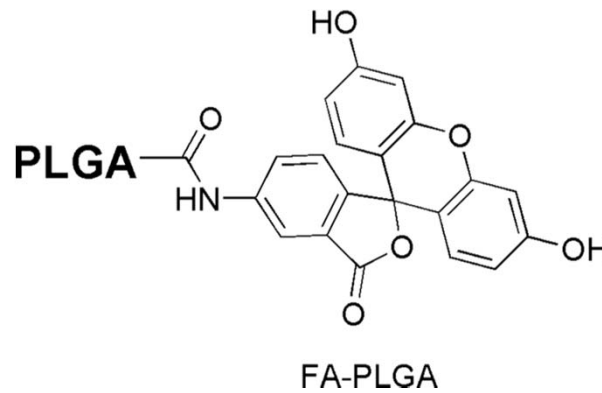


# In vivo investigations in human IBD patients

*Confocal laser endoscopy*



*Fluorescent PLGA nanoparticles*

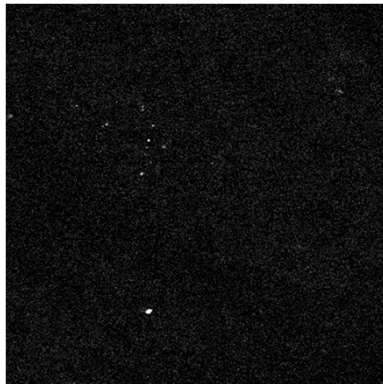


Weiss et al, *J Nanosci Nanotechnol*: 6, 1-9 (2006)



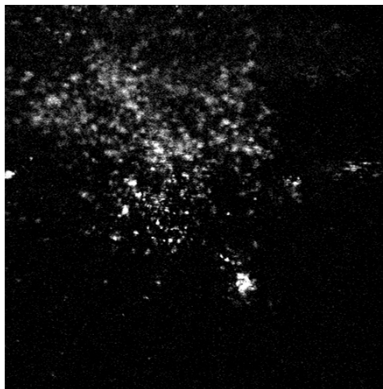
# In vivo investigations in human IBD patients

In collaboration with C. Schmidt, C. Lautenschläger, A. Stallmach, University Hospital Jena

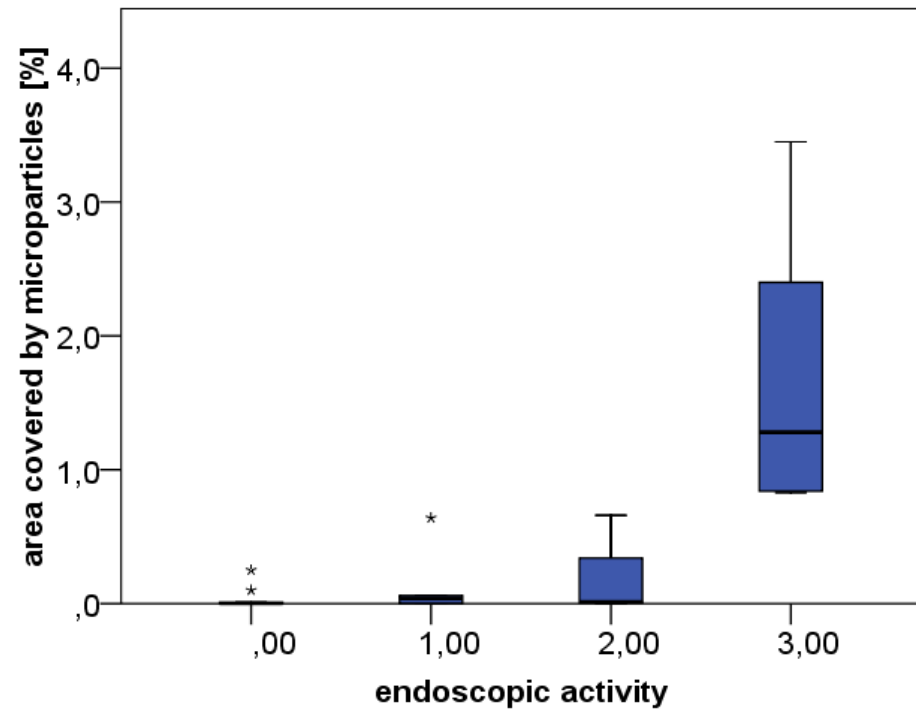


*Accumulation of FA-PLGA microparticles in the rectal mucosa of human IBD patients*

Moderately inflamed mucosa



Highly inflamed mucosa with flat ulcerations

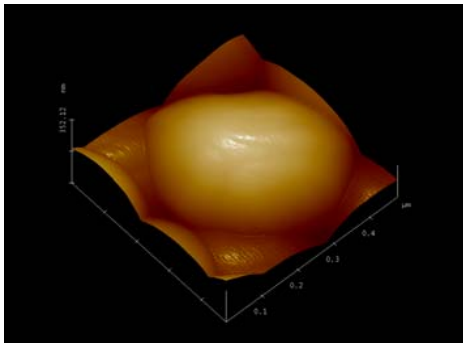


Schmidt et al, Gut, submitted

# Budesonide formulations for the treatment of IBD

In collaboration with B. Crielaard, T. Lammers, G. Storm,  
Utrecht University

Budesonide PLGA nanoparticles



size ~220 nm, PDI: 0.08  
encapsulation rate: 67  $\mu\text{g}/\text{mg}$   
encapsulation efficiency: 46%

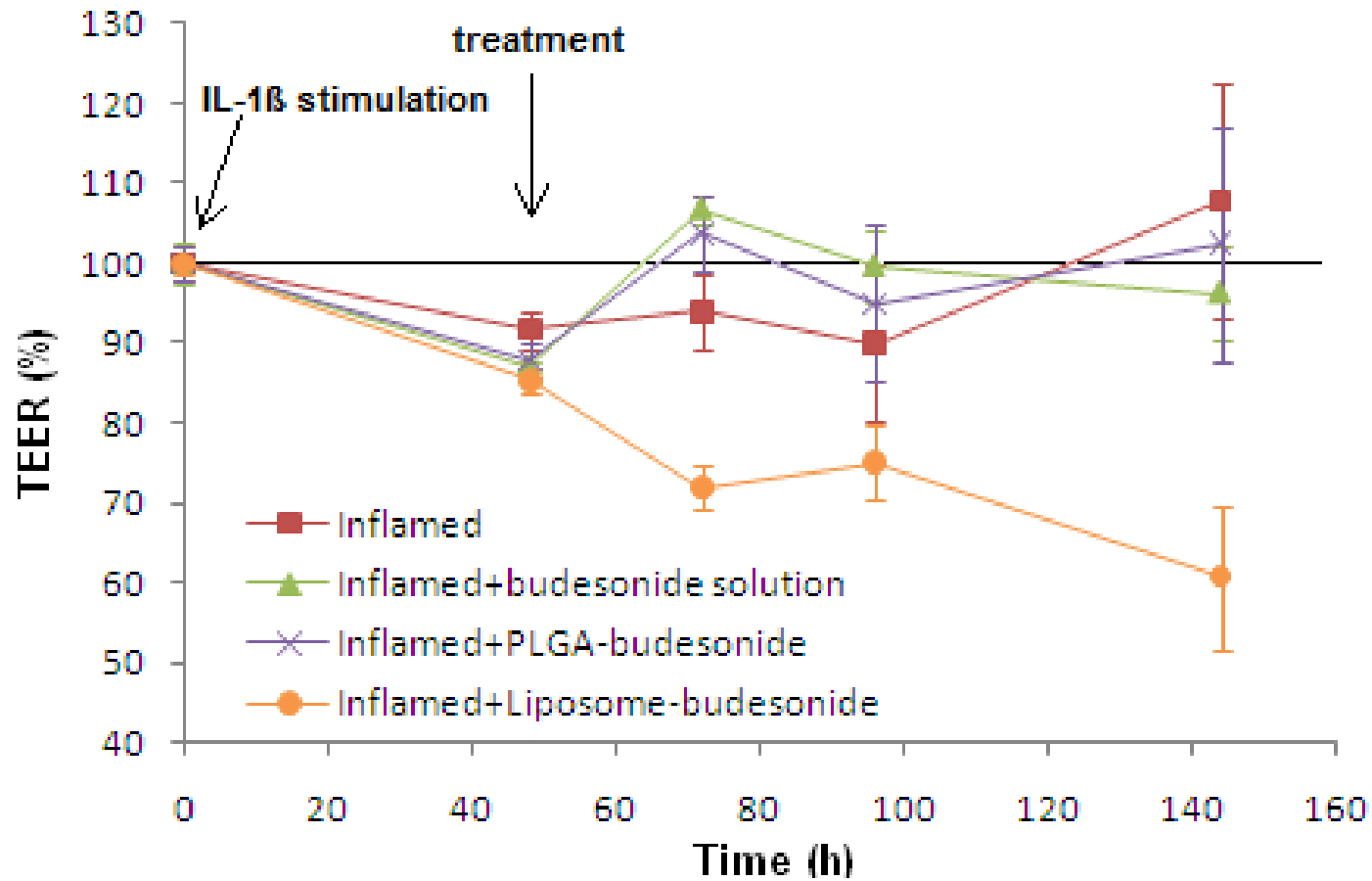
Liposomal budesonide



size ~ 200 nm, PDI: 0.05  
encapsulation rate: 4.2 mg/ml  
encapsualtion efficiency: 4.2%

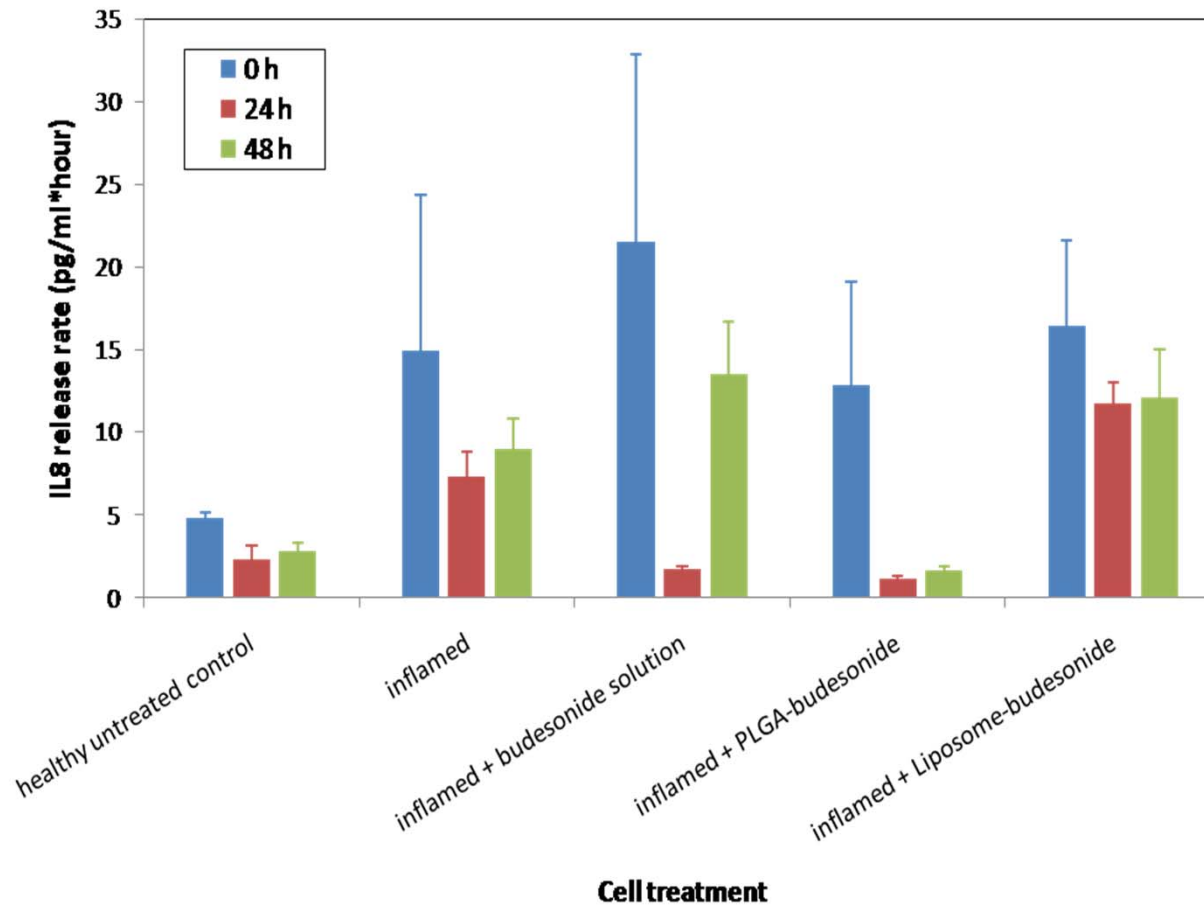
Diluted or suspended in Caco-2 medium to a concentration of **100  $\mu\text{g}/\text{ml}$**

# Testing of anti-inflammatory formulations in the inflamed 3D model



Leonard et al, EJPB, submitted

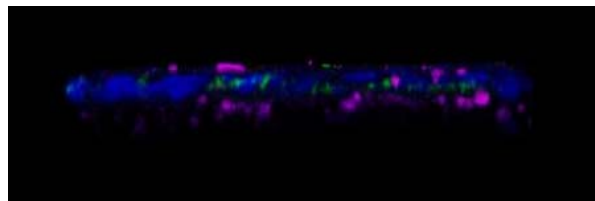
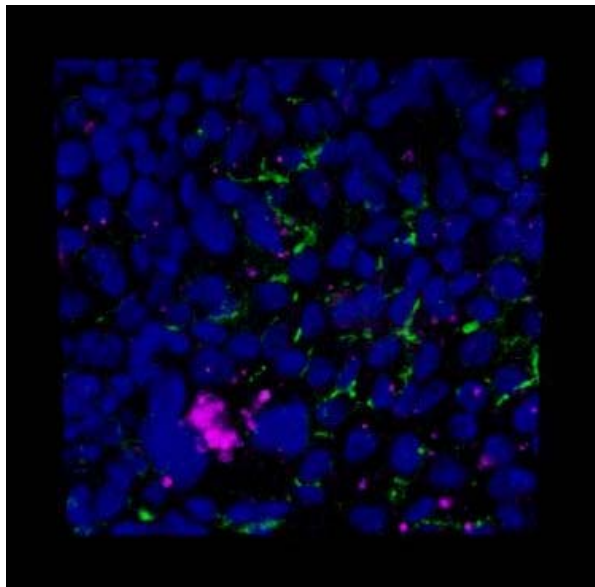
# Testing of anti-inflammatory formulations in the inflamed 3D model



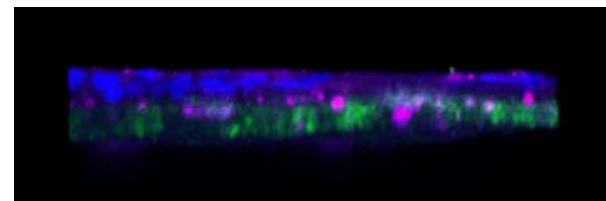
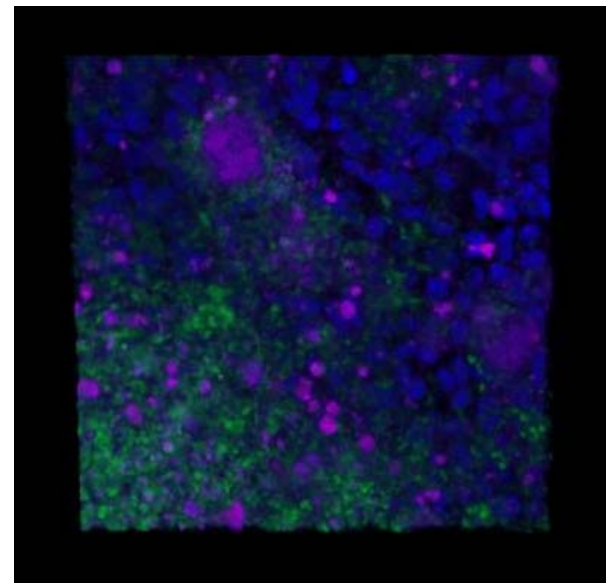
Leonard et al, EJPB, submitted

# Testing of anti-inflammatory formulations in the inflamed 3D model

*Budesonide PLGA nanoparticles*

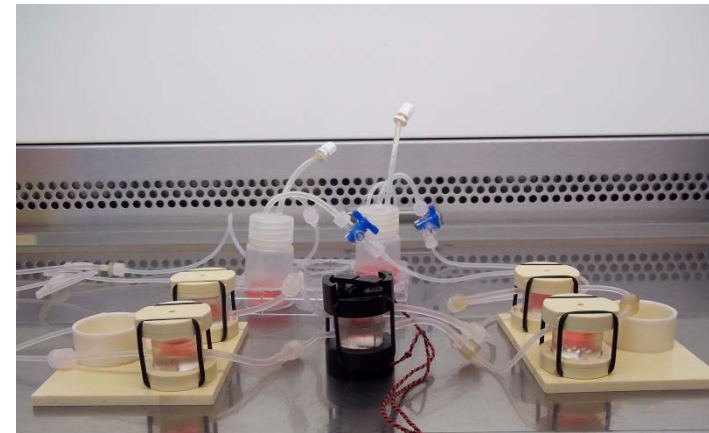
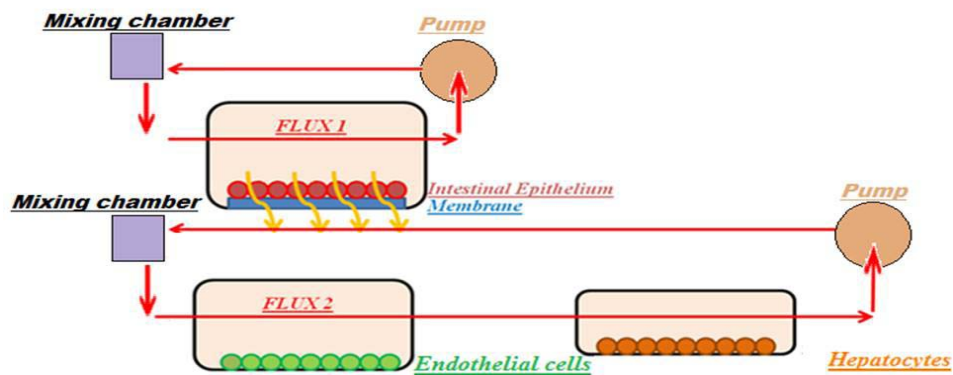


*Liposomal budesonide*



*Leonard et al, EJPB, submitted*

# Other applications of the 3D model of the inflamed intestinal mucosa: nanotoxicology



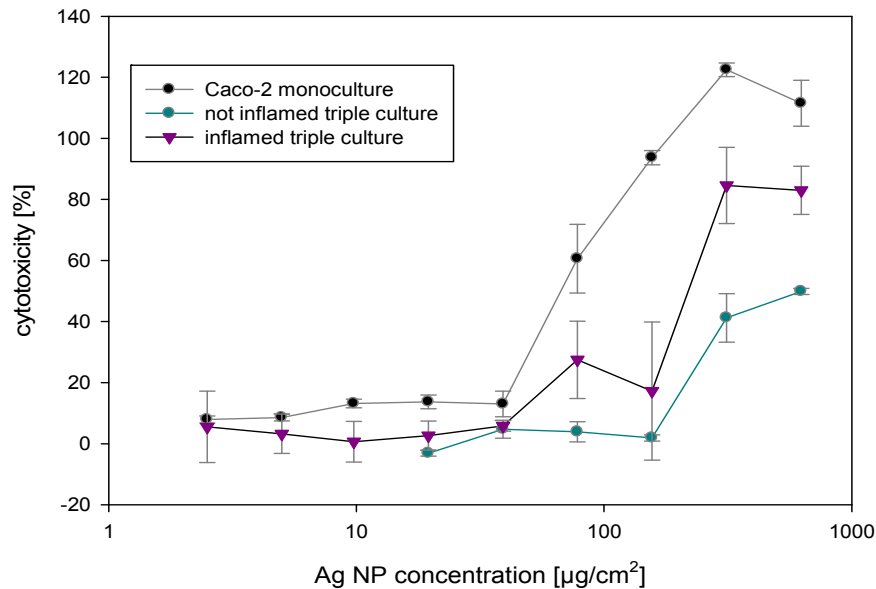
Interaction of the susceptible, inflamed intestinal barrier with (engineered) nanoparticles and other xenobiotics

Particle translocation and downstream signaling to endothelial cells and hepatocytes

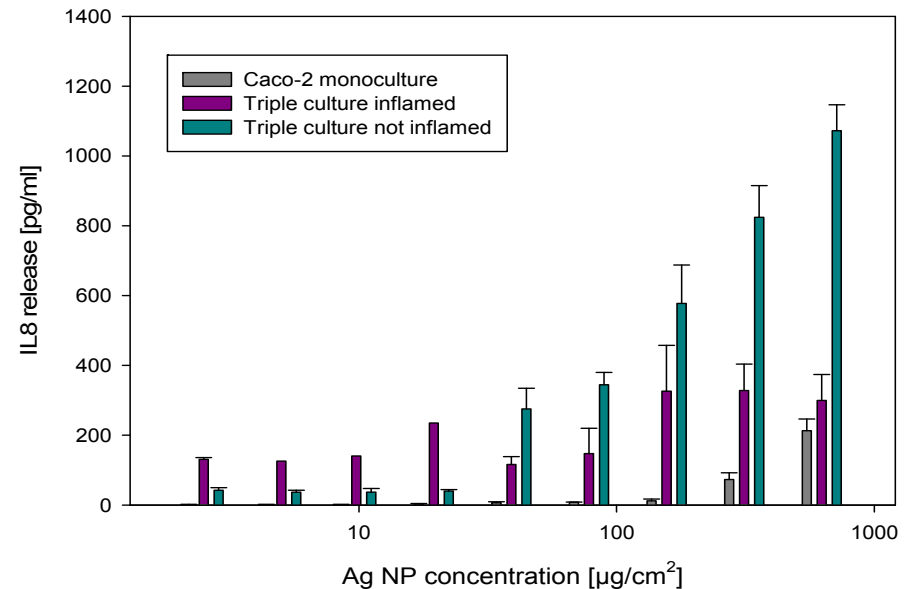


# Other applications of the 3D model of the inflamed intestinal mucosa: nanotoxicology

Significant change in response pattern compared to single culture:

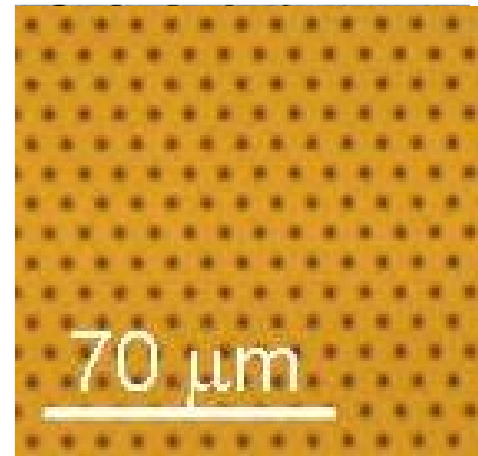
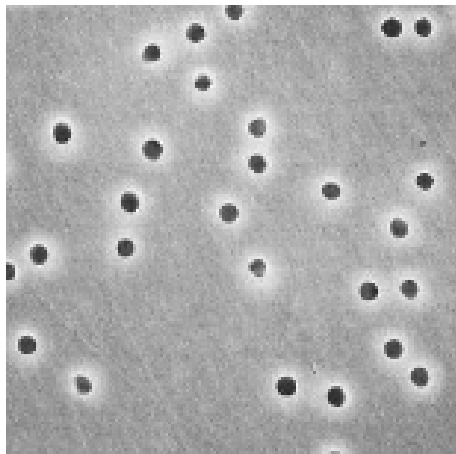
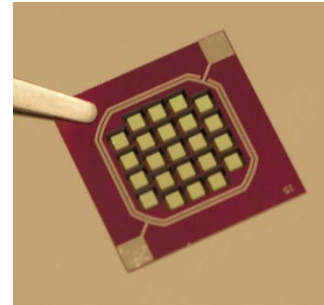


Reduced epithelial damage

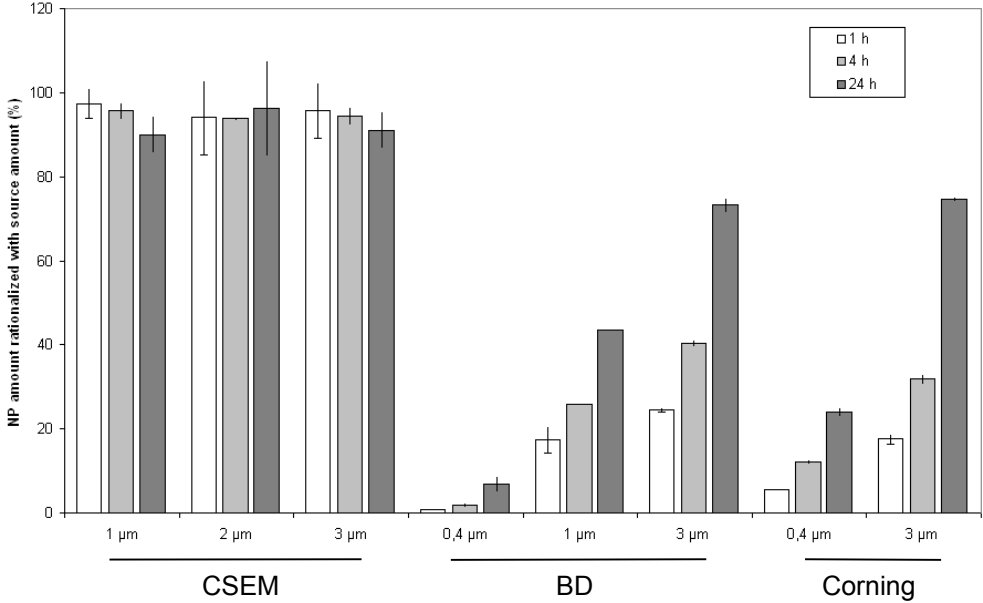
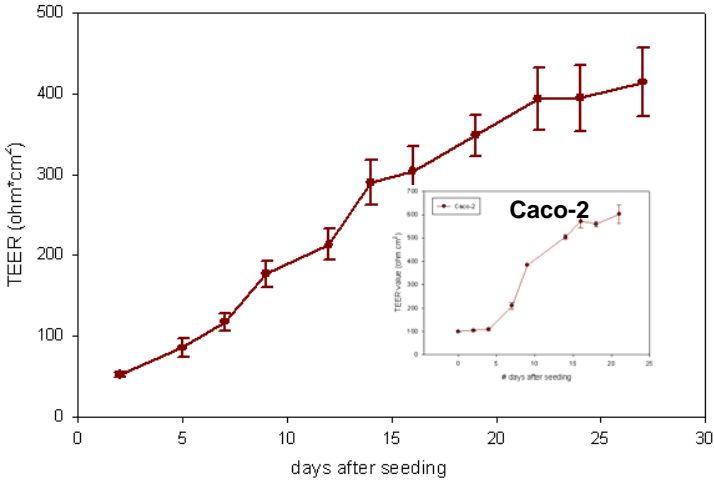
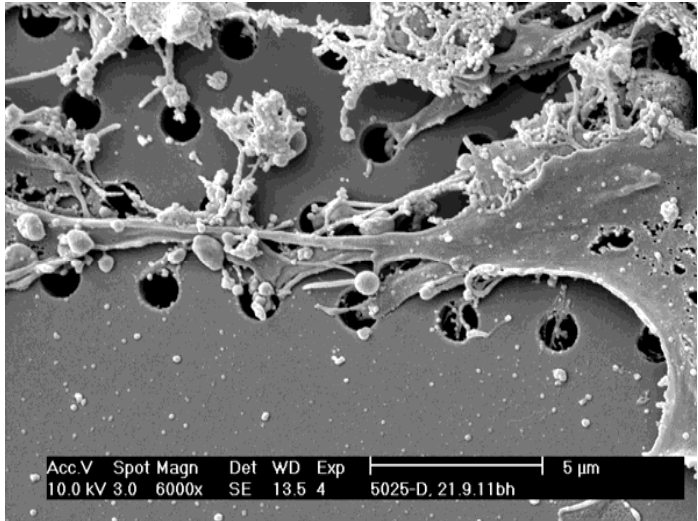


Increased inflammatory reaction

# It's only a matter of support: new directions for advanced intestinal cell models



# It's only a matter of support: new directions for advanced intestinal cell models



Helmholtz-Institut für Pharmazeutische Forschung Saarland



# Summary

- Successful establishment of a novel cell culture model simulating the intestinal mucosa in the state of inflammation
- Pathophysiological changes reflected in the model: release of pro-inflammatory markers, activation of immune cells, decreased barrier function, re-organization of tight junctions, increased mucus production
- Applications of the model:
  - anti-inflammatory drug and formulation testing in pharmaceutical development
  - investigation of the interaction of (engineered) nanoparticles or other xenobiotics with the susceptible barrier
- Advantages over existing animal models: ethical aspect, no species differences, similar pathogenesis, mechanistical insight, cost and time reduction

# Acknowledgements



## Financial support



Helmholtz-Institut für Pharmazeutische Forschung Saarland





# 9<sup>th</sup>

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