

In vitro co-culture model of the inflamed intestinal mucosa

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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- α)

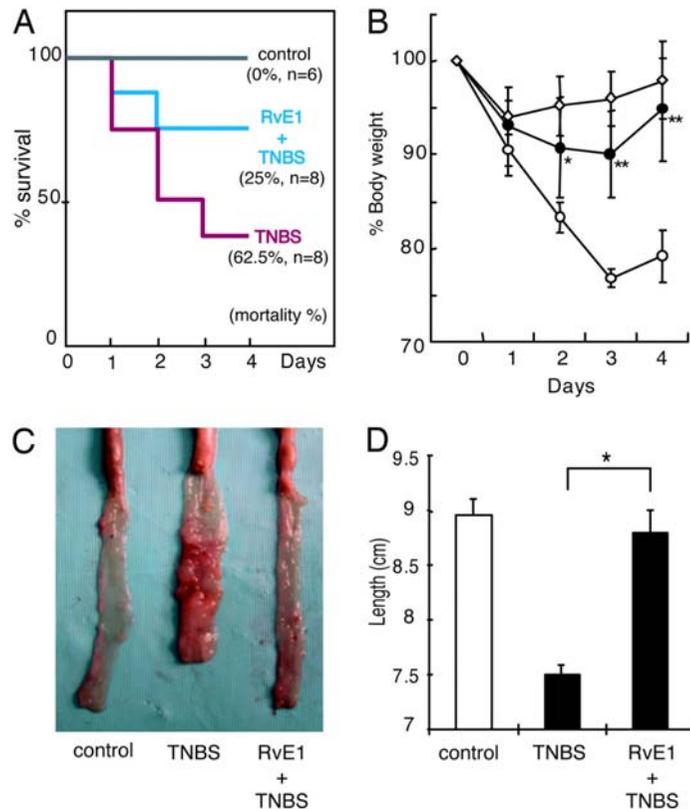


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State of the art: animal models in drug/formulation development for IBD treatment

Rodent colitis models

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



Symptoms:

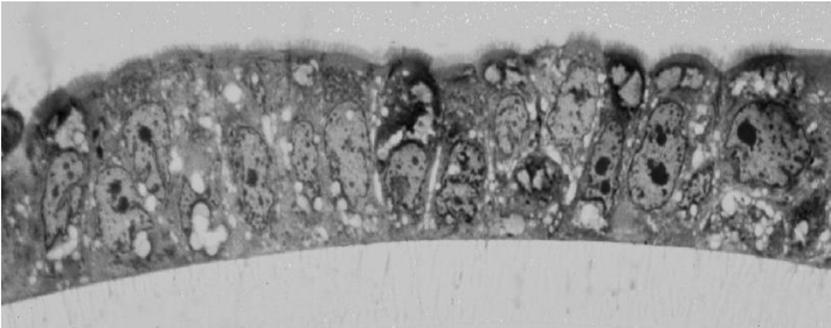
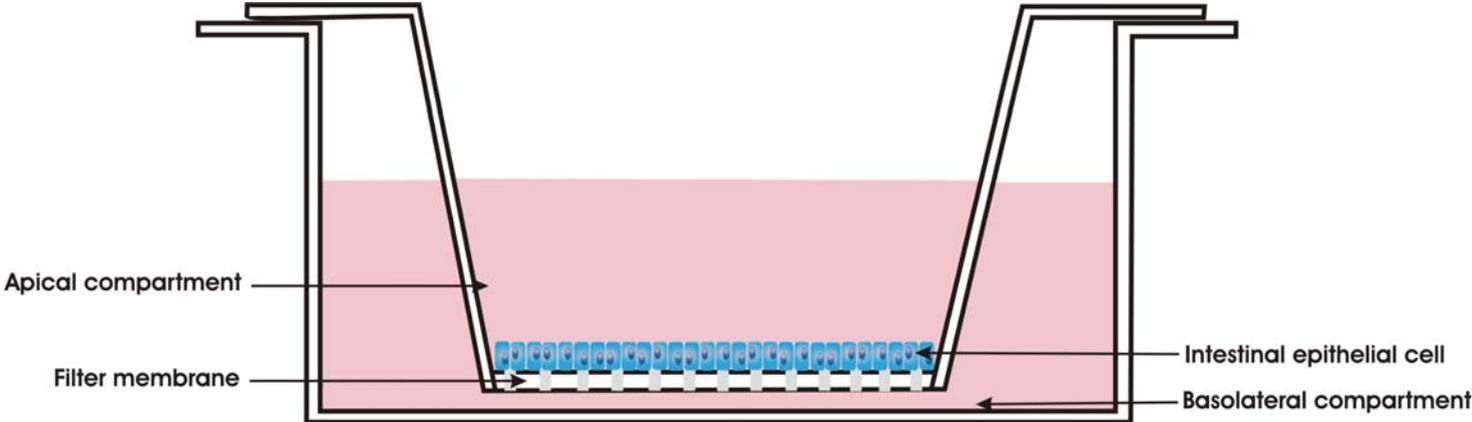
Diarrhea, rectal bleeding, weight loss, pain, colon perforation, sepsis, death

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

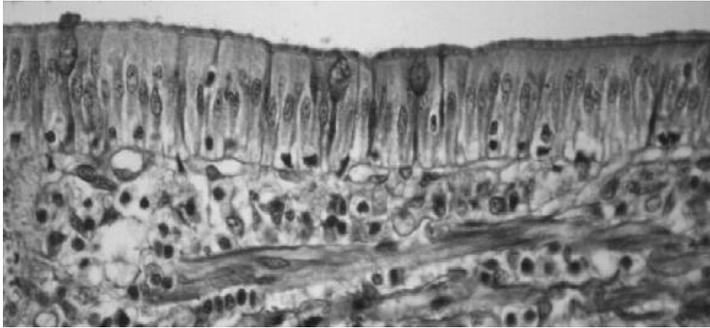
Issues: unethical, differences in species and pathogenesis

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

In vitro test systems for oral bioavailability

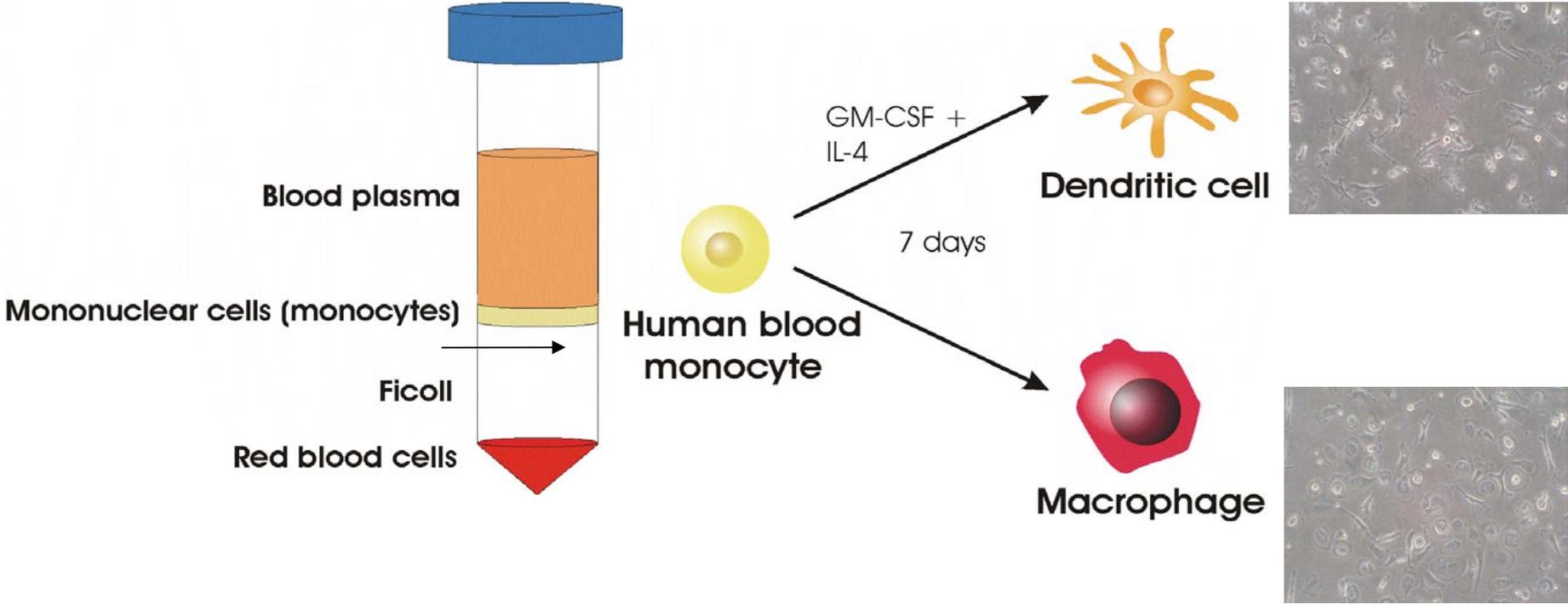


Caco-2 monolayer

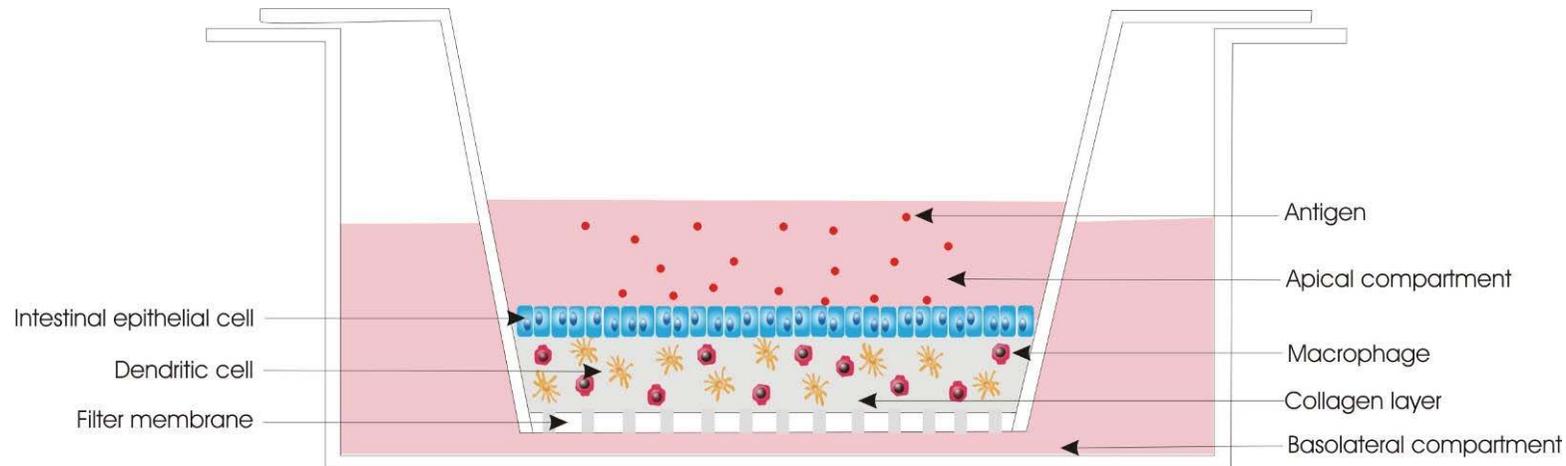


Intestinal mucosa

Adding complexity: immune cells



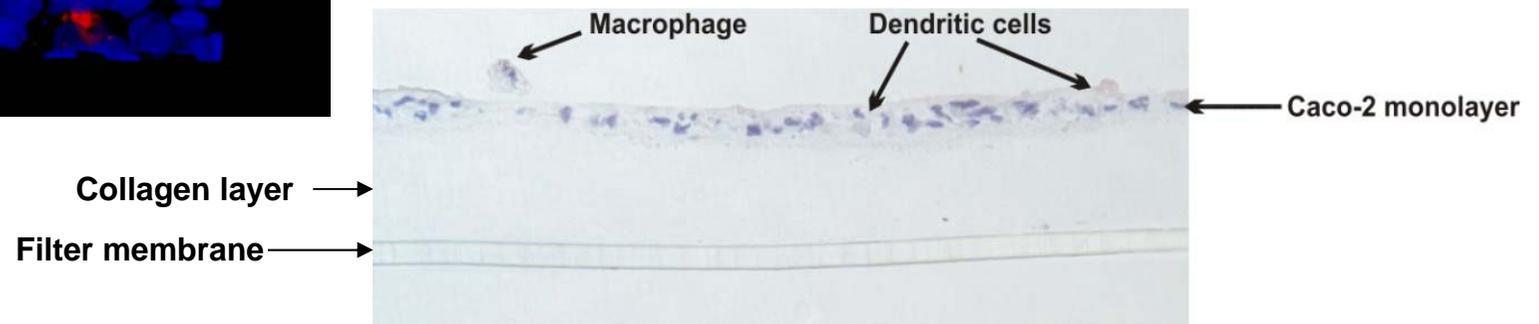
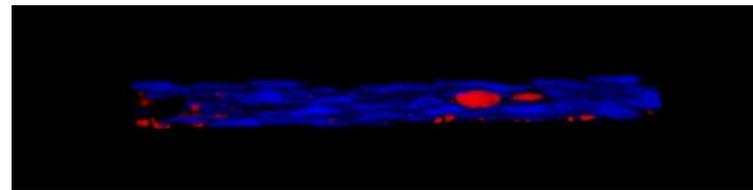
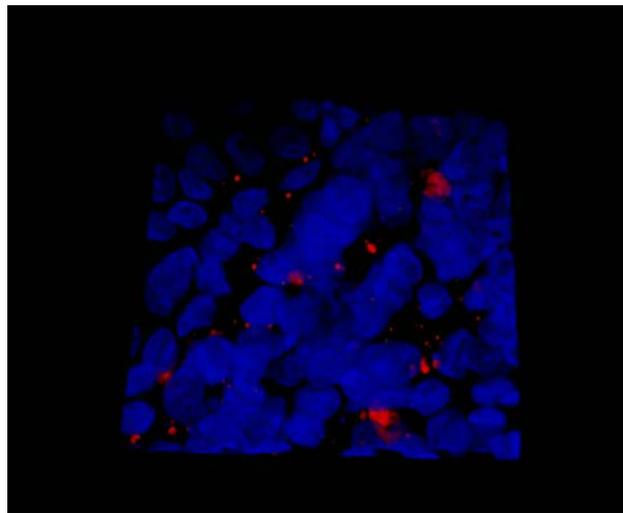
3D in vitro model of the inflamed intestinal mucosa



- 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 β) to the cell culture medium
- Should reflect the relevant pathophysiological changes occurring in vivo: release of pro-inflammatory markers (IL-8, TNF- α), re-organisation of tight junctional proteins, reduced barrier function, increased mucus production

Pathophysiological changes in the 3D model

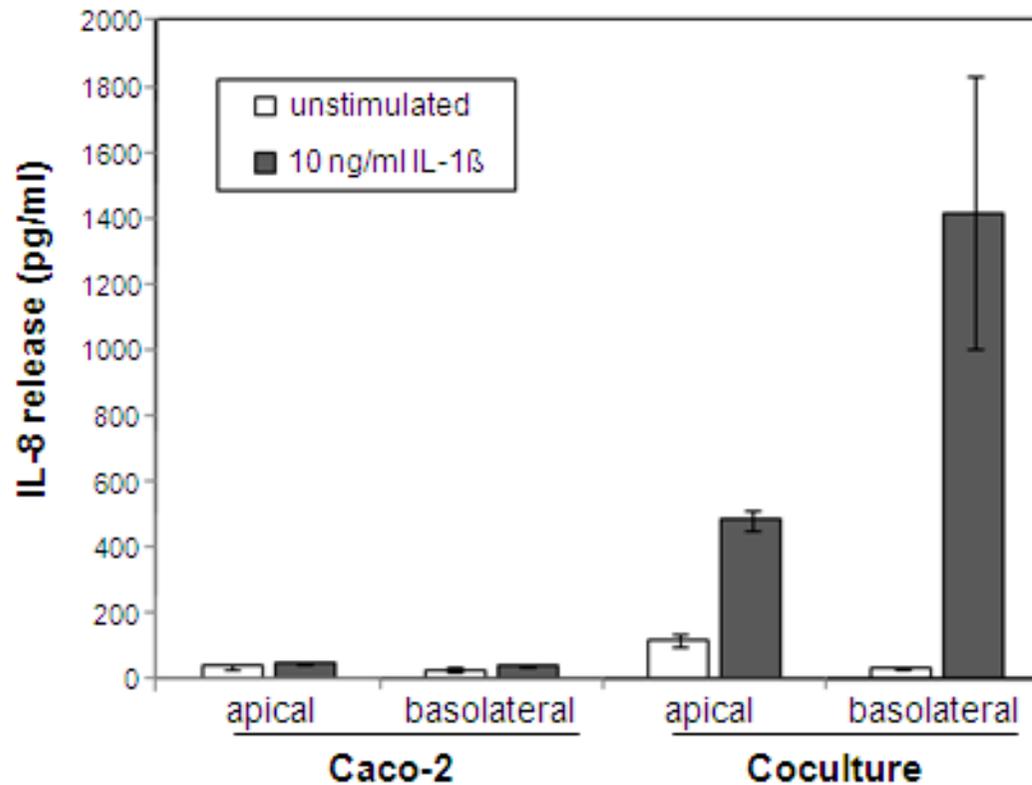
*Infiltration of immunocompetent cells
(macrophages + dendritic cells)*



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

Pathophysiological changes in the 3D model

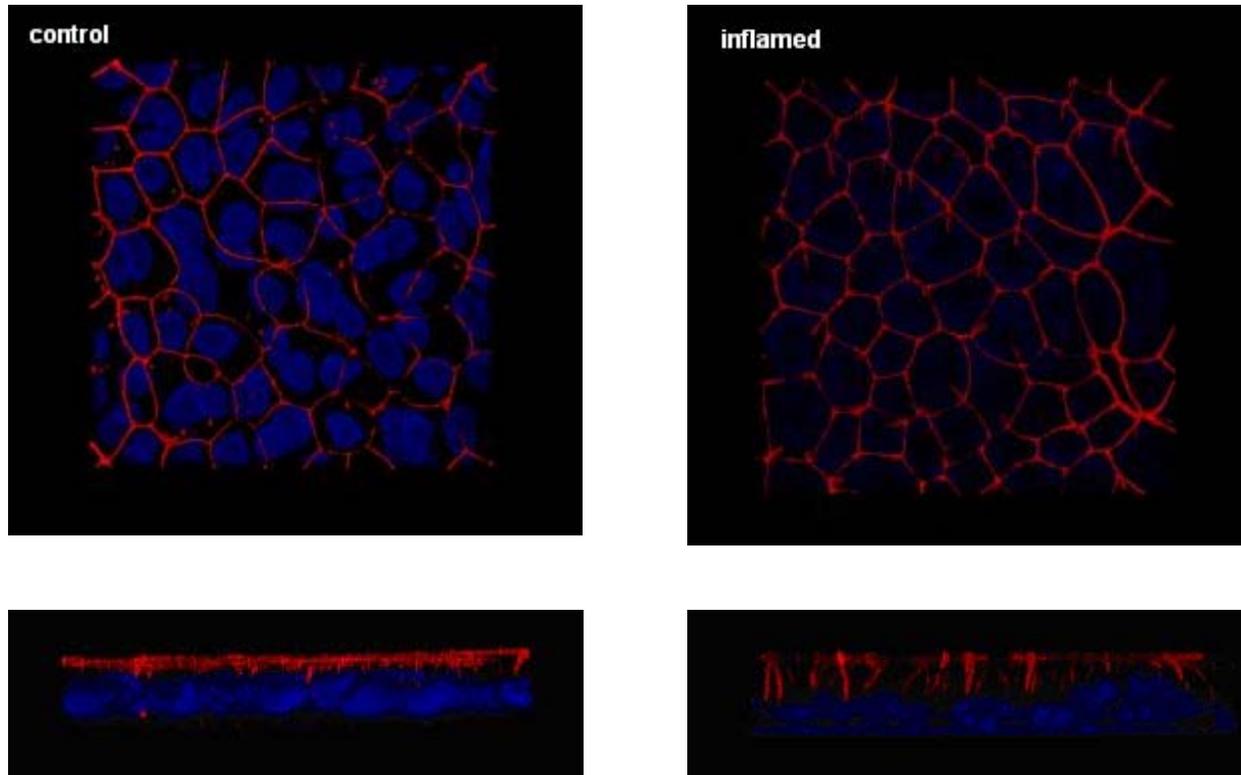
Upregulation and release of pro-inflammatory markers, e.g. IL-8 or TNF- α



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

Pathophysiological changes in the 3D model

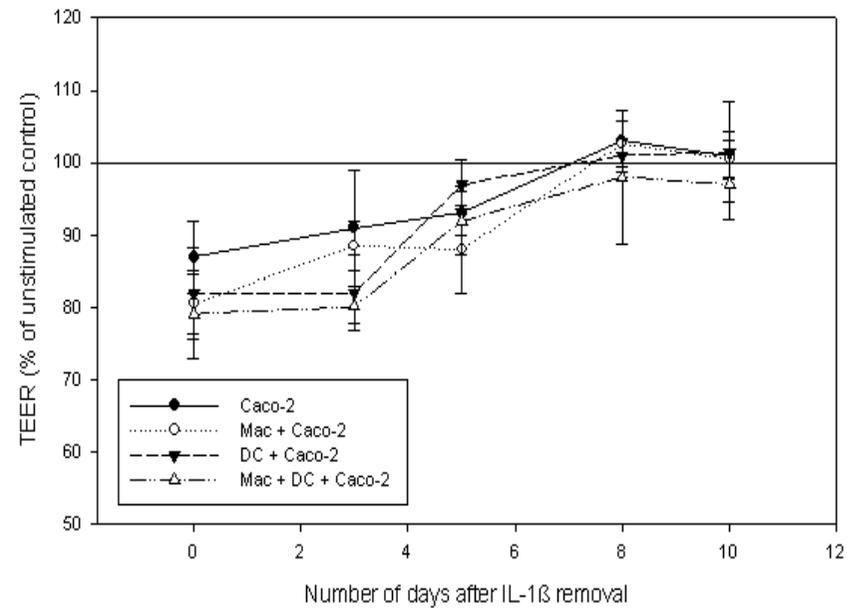
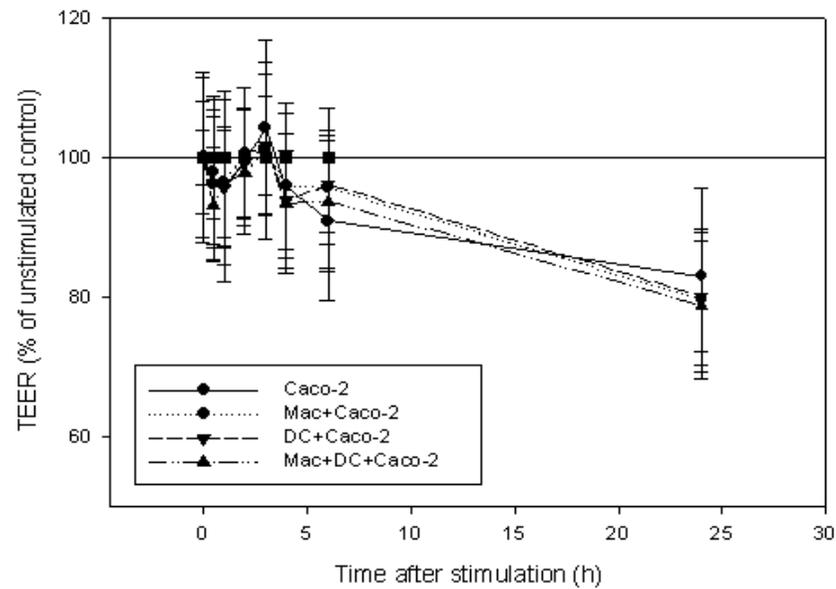
Changes in tight junctional organization (ZO-1) ...



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

Pathophysiological changes in the 3D model

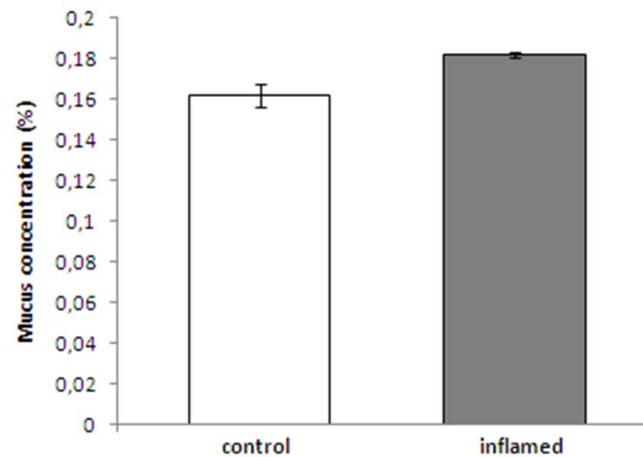
... and barrier function



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

Pathophysiological changes in the 3D model

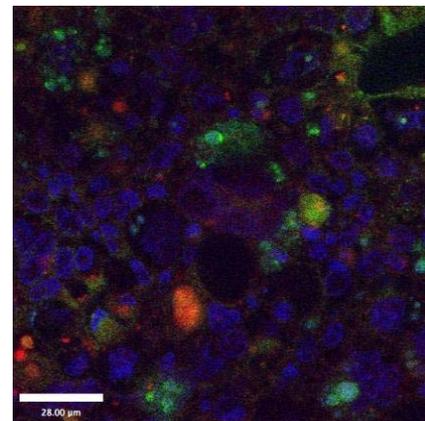
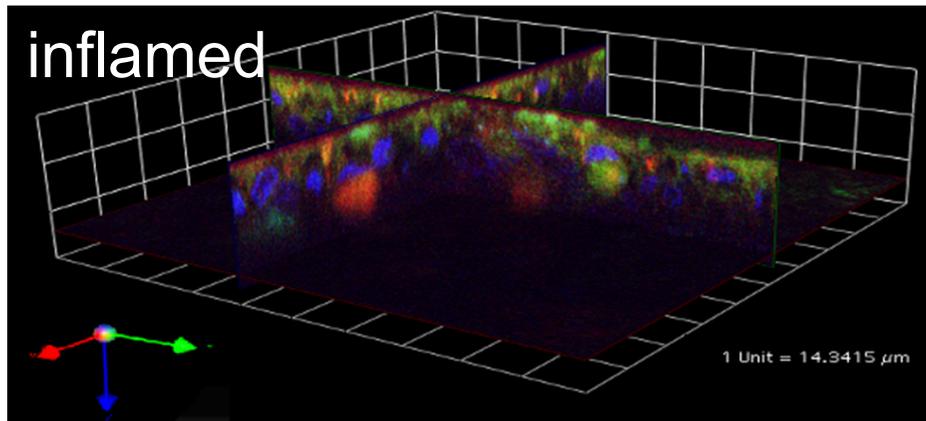
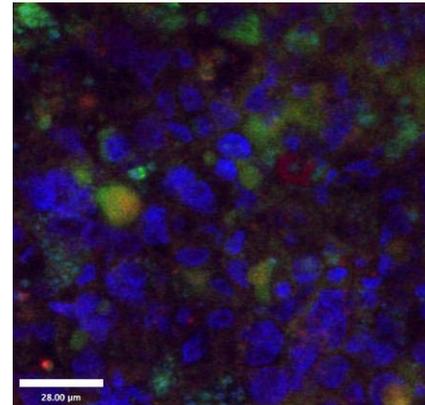
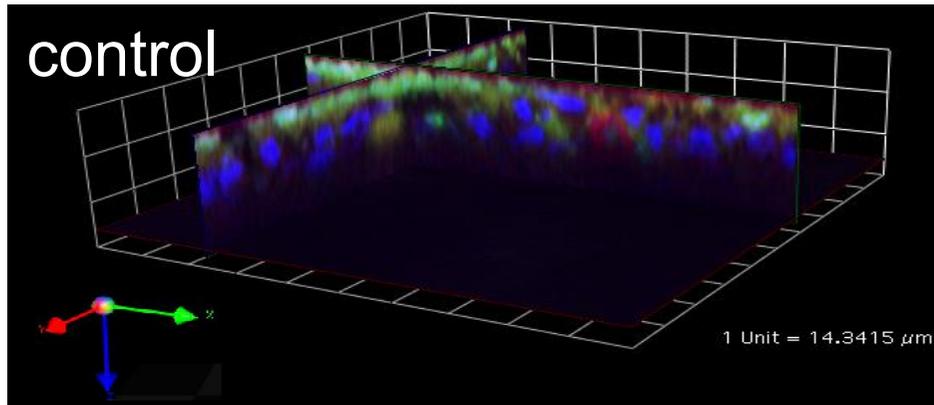
Increased mucus production



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

Pathophysiological changes in the 3D model

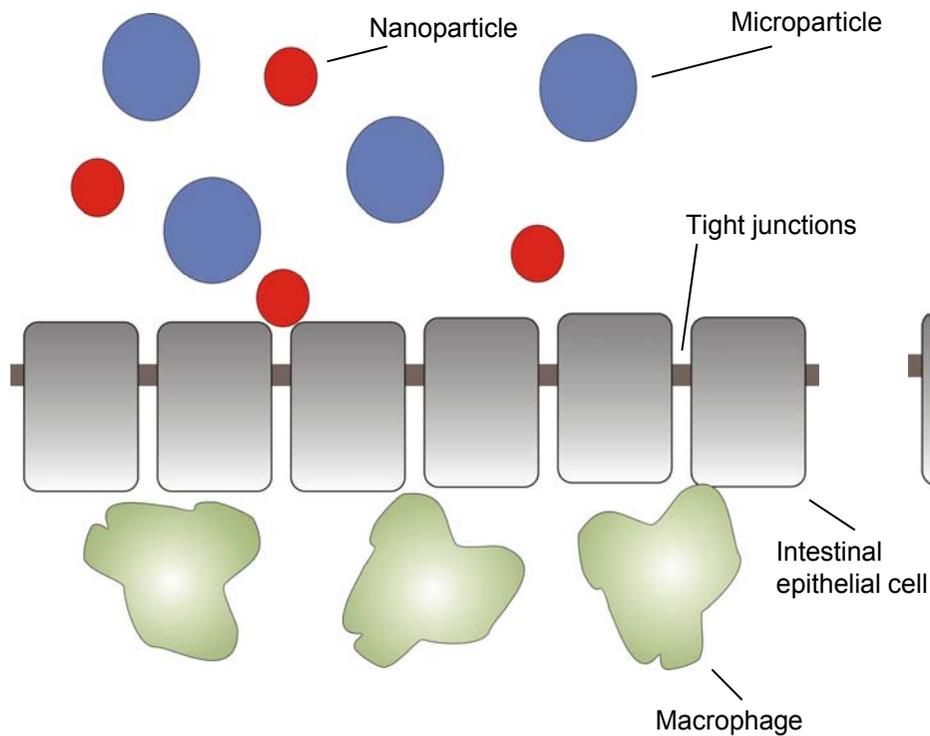
Increased activity of immune cells



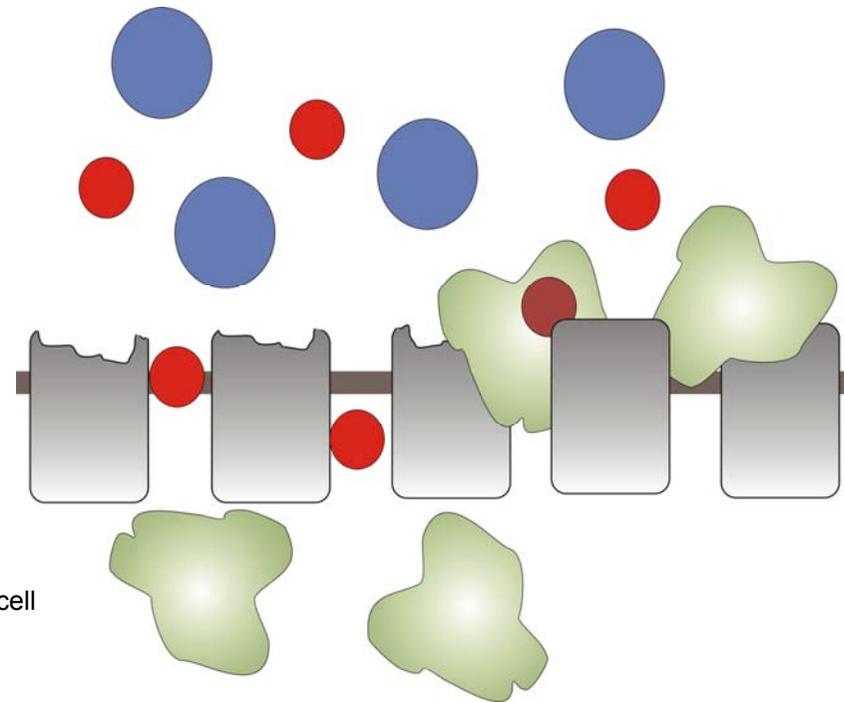
Leonard et al, Mol Pharm: 7(6), 2103-19 (2010)

Pathophysiological changes in the inflamed mucosa: Threat or potential?

Healthy mucosal barrier

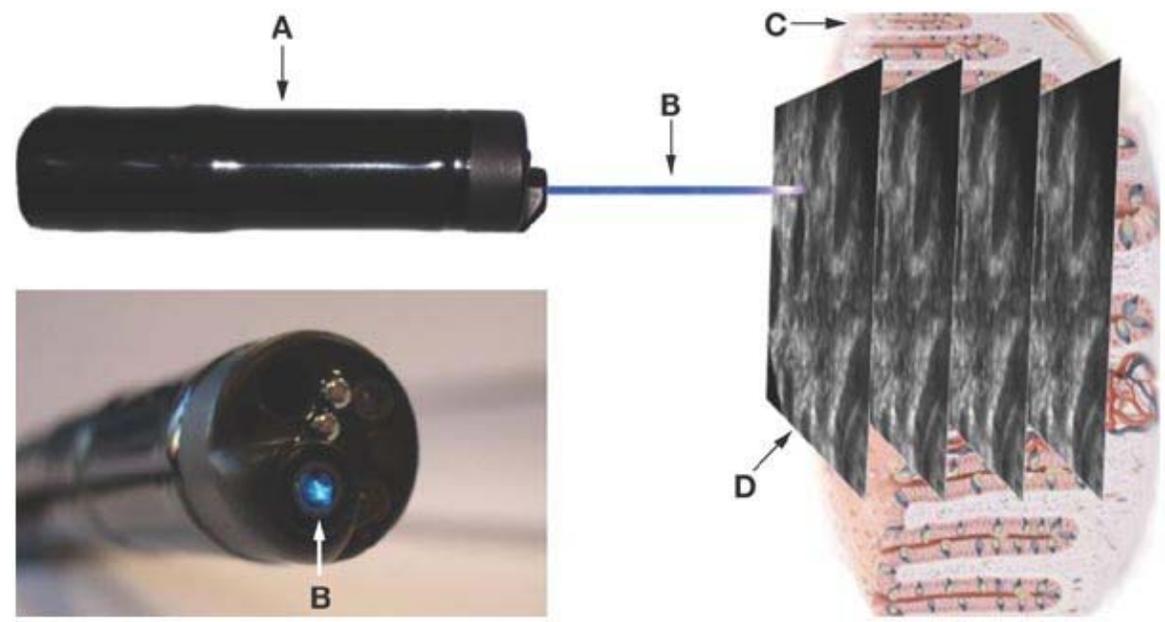


Inflamed mucosal barrier

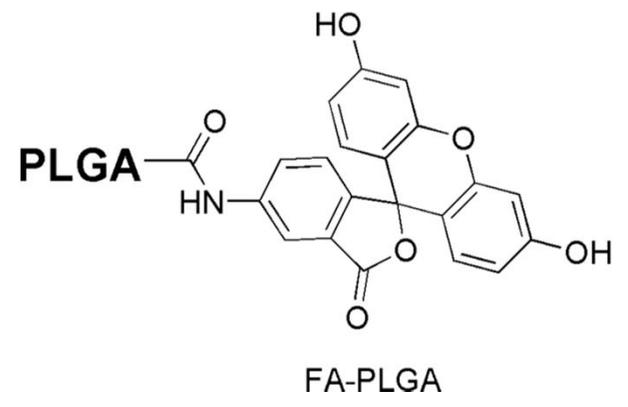


In vivo investigations in human IBD patients

Confocal laser endoscopy



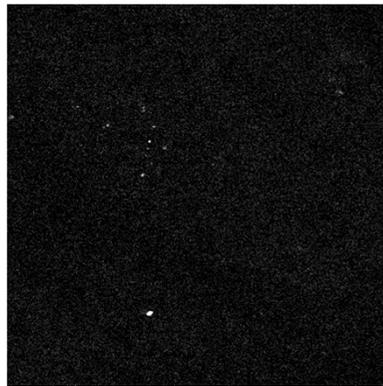
Fluorescent PLGA nanoparticles



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

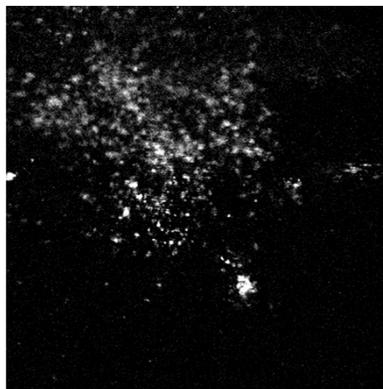
In vivo investigations in human IBD patients

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

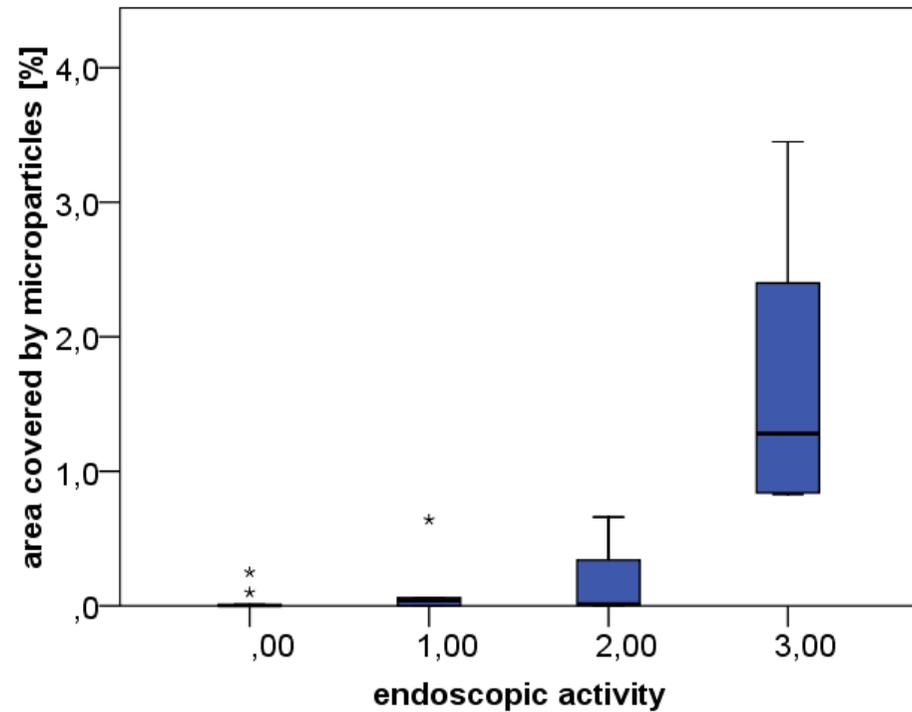


Accumulation of FA-PLGA microparticles in the rectal mucosa of human IBD patients

Moderately inflamed mucosa



Highly inflamed mucosa with flat ulcerations

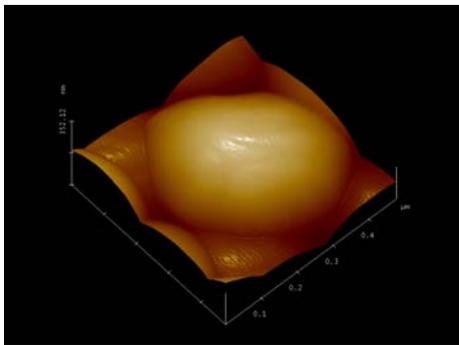


Schmidt et al, Gut, submitted

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%

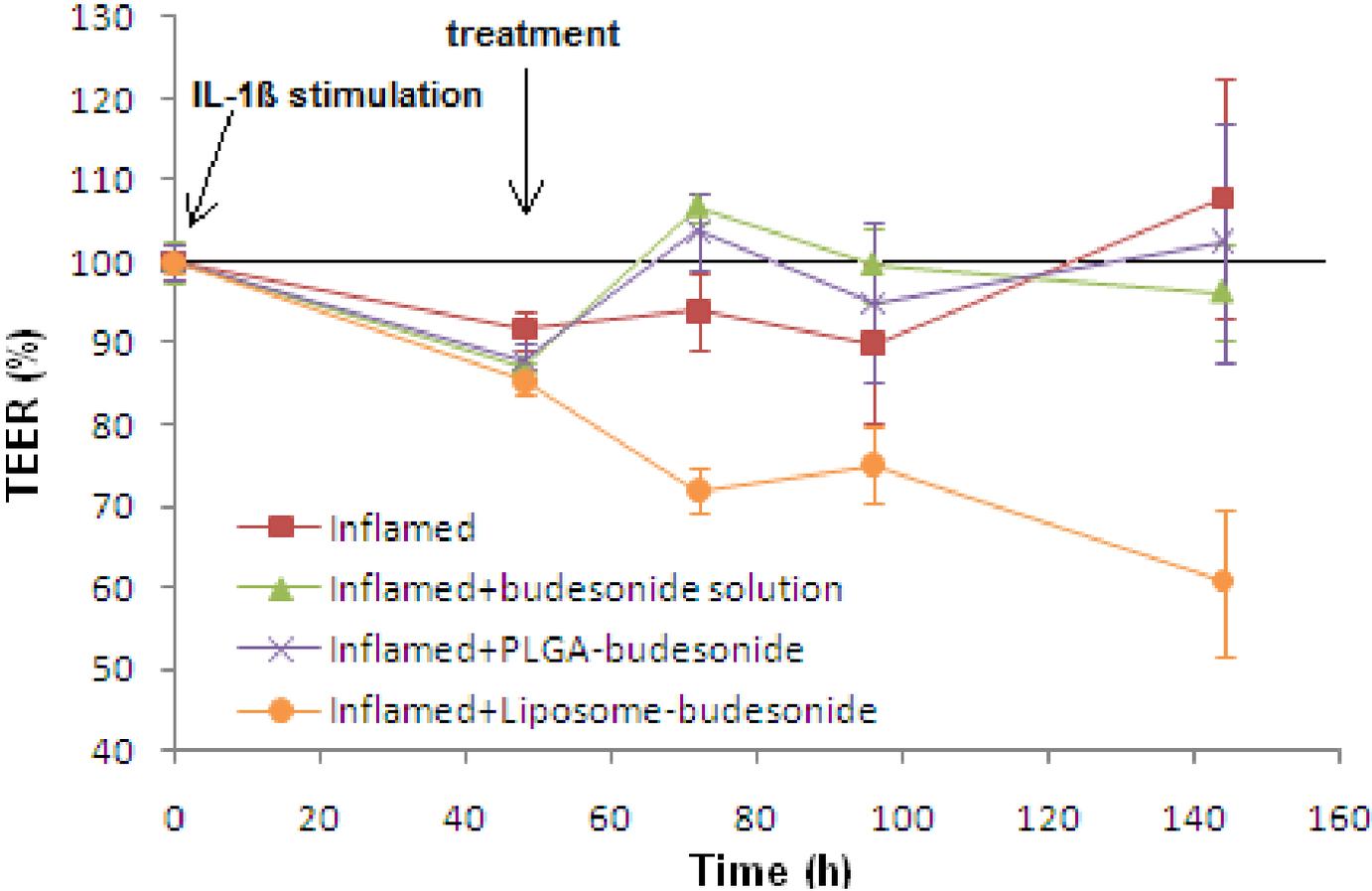
Liposomal budesonide



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

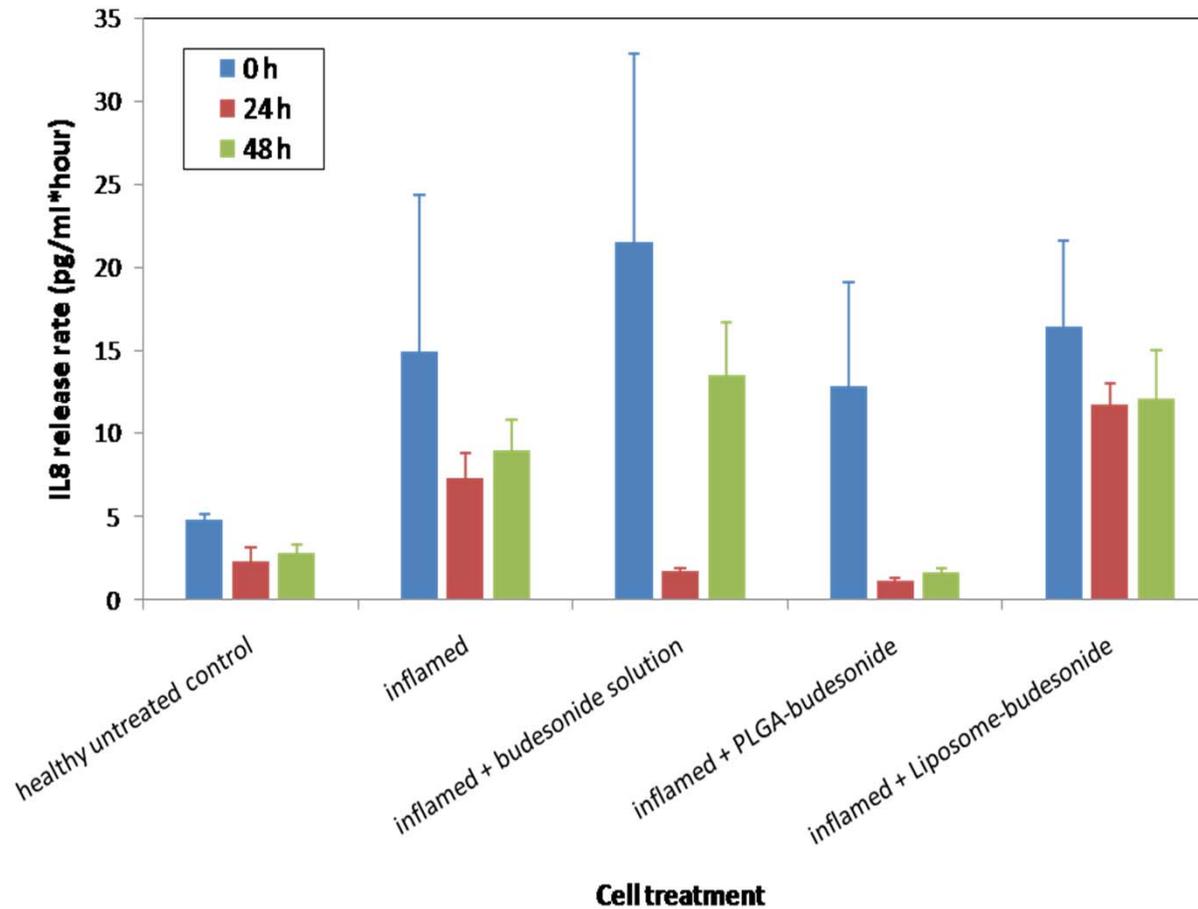
Diluted or suspended in Caco-2 medium to a concentration of **100 $\mu\text{g}/\text{ml}$**

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



Leonard et al, EJPB, submitted

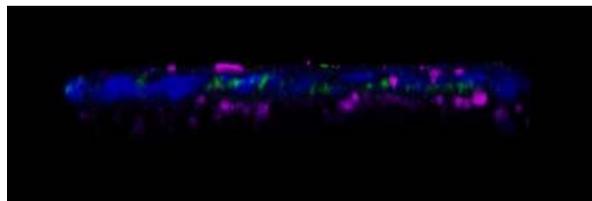
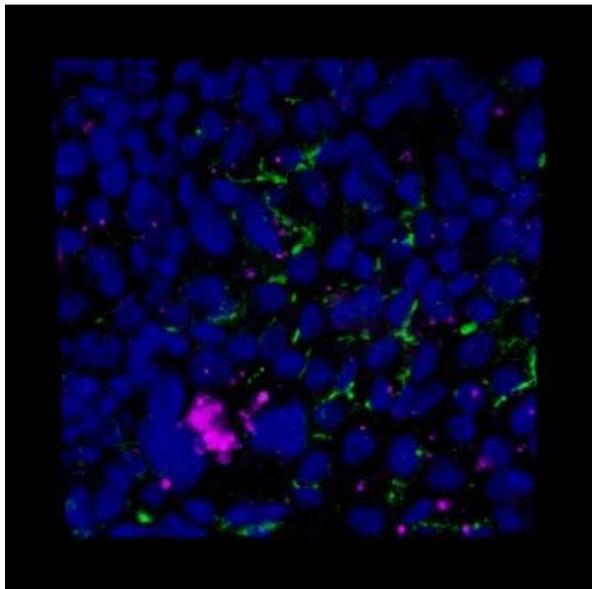
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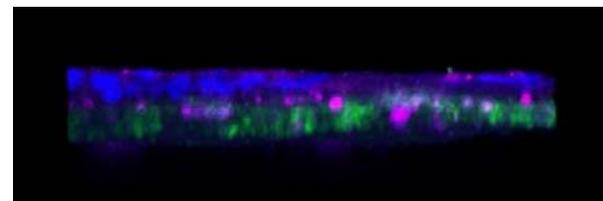
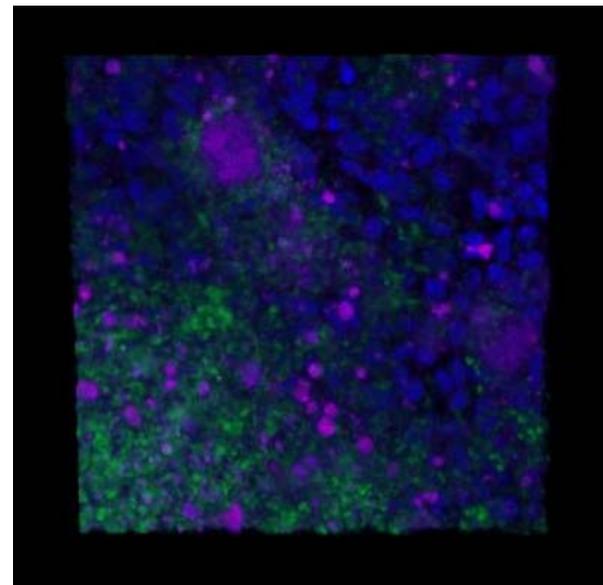
Leonard et al, EJPB, submitted

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

Budesonide PLGA nanoparticles

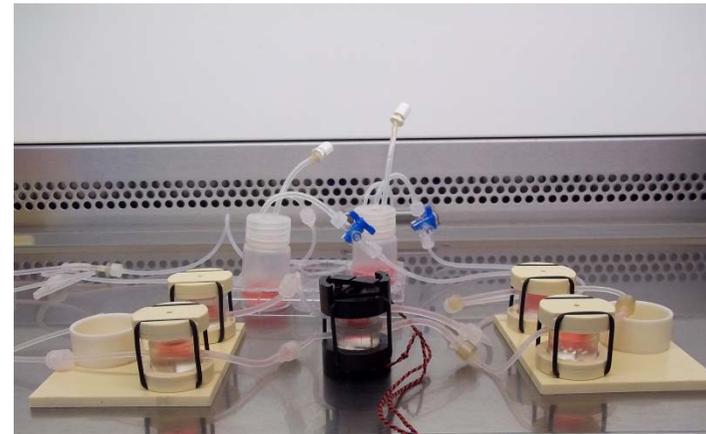
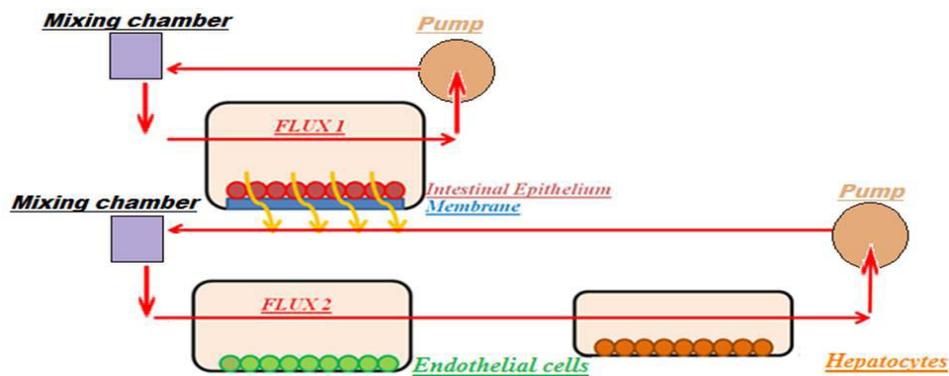


Liposomal budesonide



Leonard et al, EJPB, submitted

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

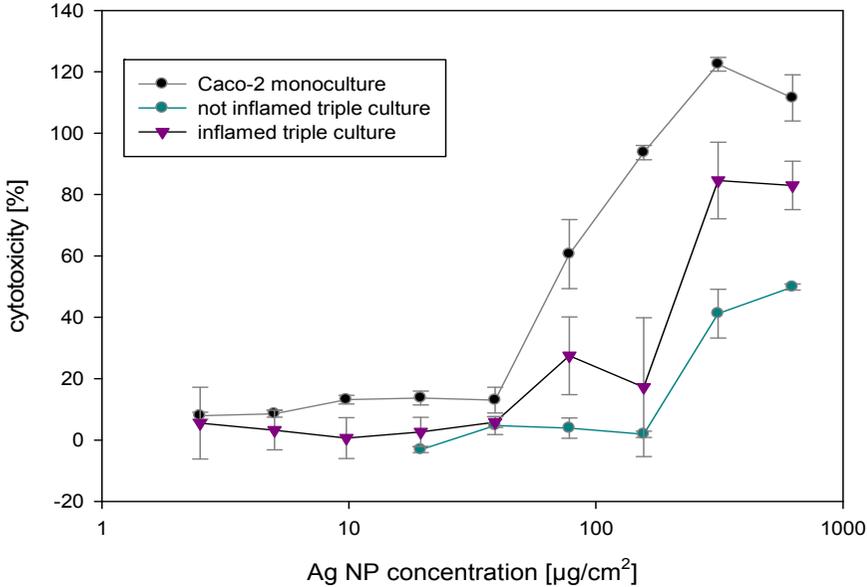


Interaction of the susceptible, inflamed intestinal barrier with (engineered) nanoparticles and other xenobiotics

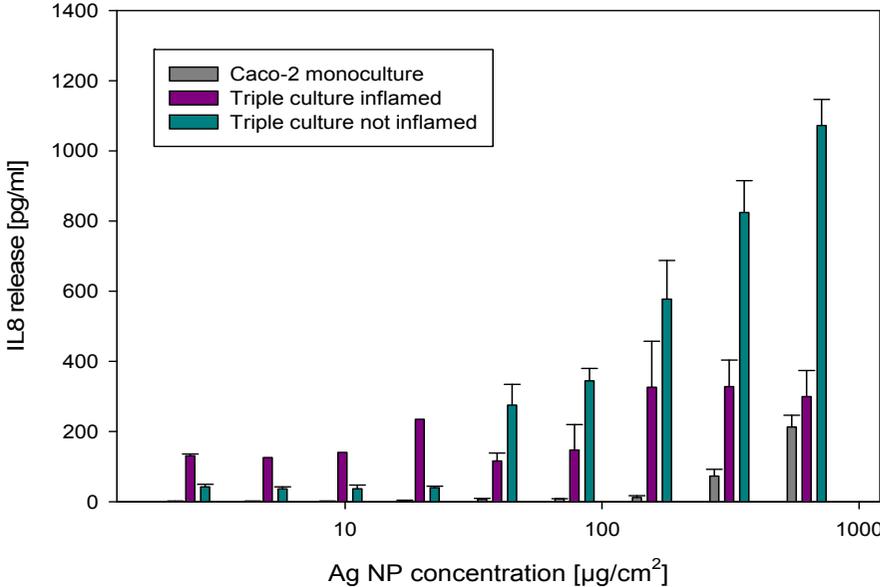
Particle translocation and downstream signaling to endothelial cells and hepatocytes

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

Significant change in response pattern compared to single culture:

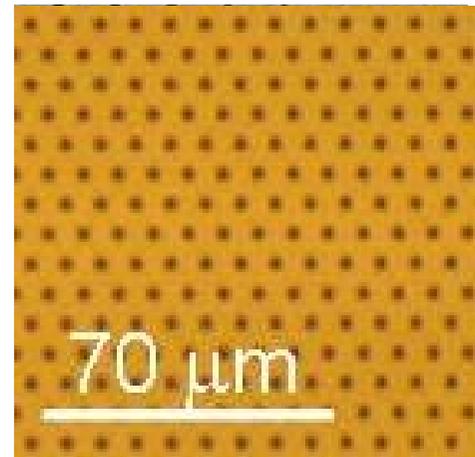
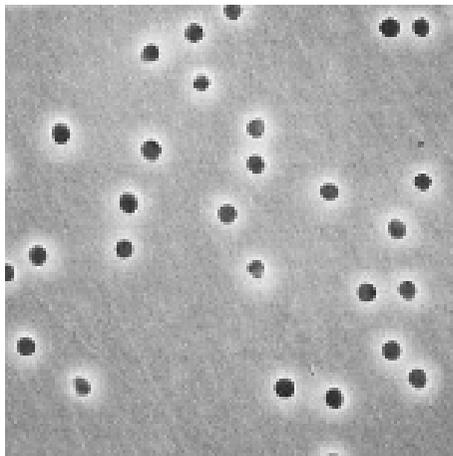
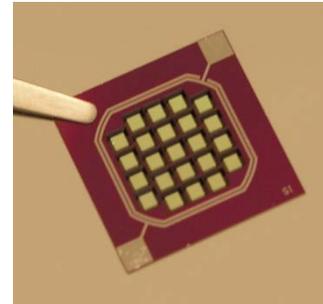


Reduced epithelial damage

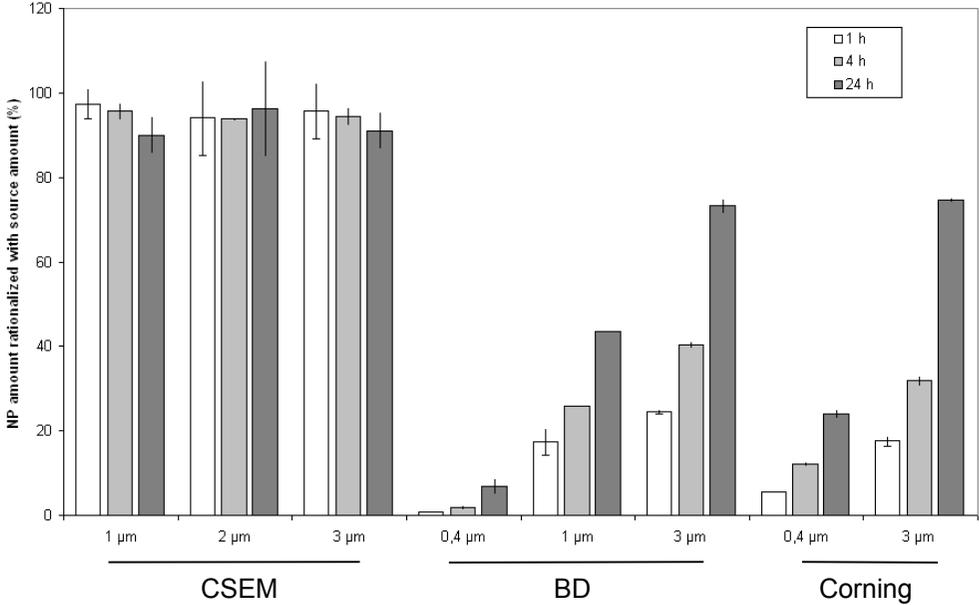
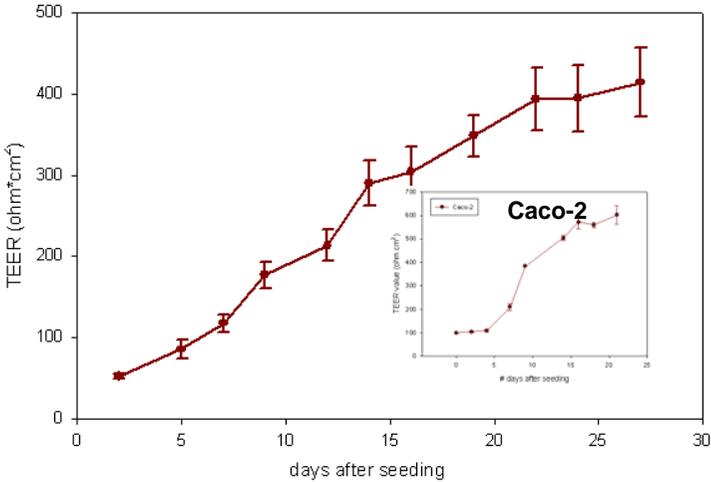
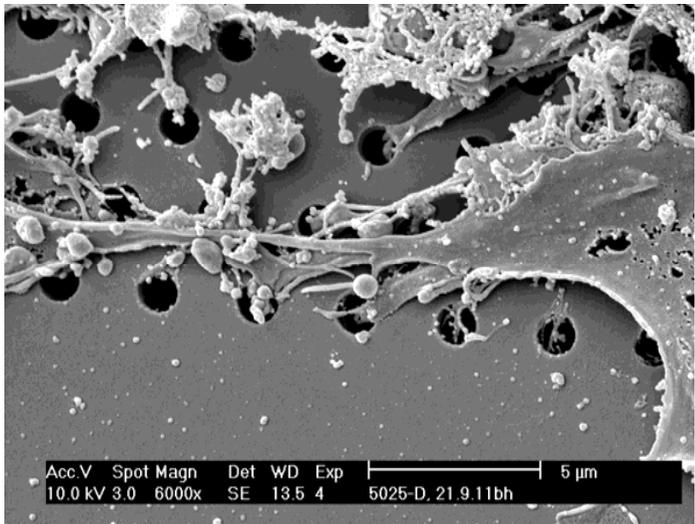


Increased inflammatory reaction

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



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



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Helmholtz-Institut für Pharmazeutische Forschung Saarland



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