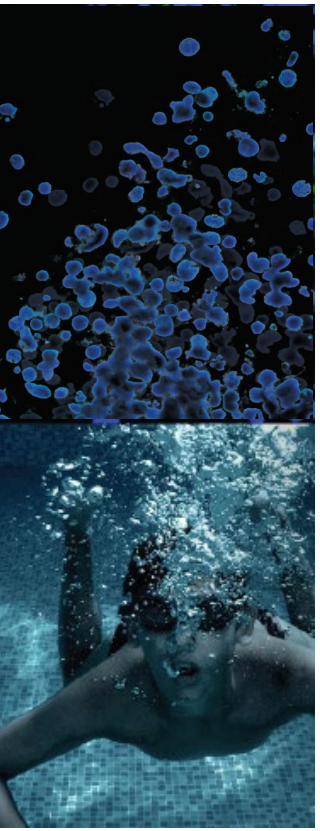
Does All Disease Begin in the Gut?
Monitoring the Barrier Function of an In Vitro Gut Mimic



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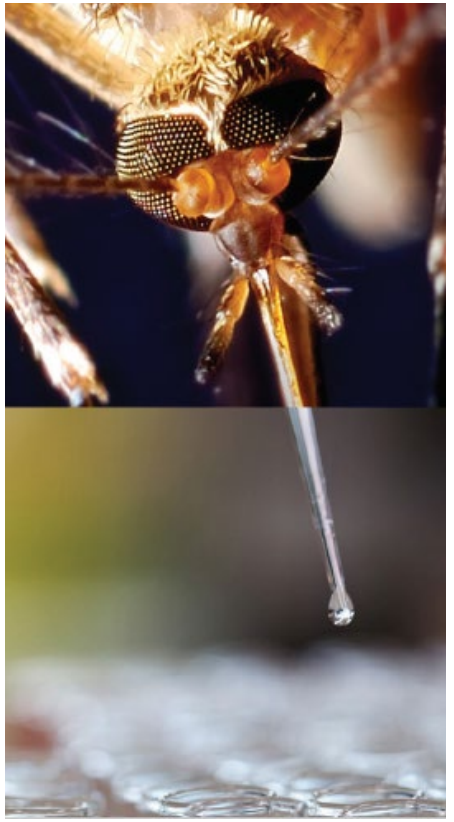
Locsense B.V.

Credible Leads to Incredible™



About ATCC

- Founded in 1925, ATCC is a non-profit organization with HQ in Manassas, VA, and an R&D and Services center in Gaithersburg, MD
- World's largest, most diverse biological materials and information resource for cell culture – the "*gold standard*"
- Innovative R&D company featuring advanced models, differentiated stem cells, gene editing
- Partner with government, industry, and academia
- Leading global supplier of authenticated cell lines, viral and microbial standards
- Sales and distribution in 150 countries, 19 international distributors
- Talented team of 550+ employees, over one-third with advanced degrees

ATCC®

Does all disease begin in the gut?

~Hippocrates

Monitoring the barrier function of an in vitro gut mimic

Eline Geervliet, MSc
Application scientist at Locsense B.V.
www.Locsense.nl

AST APPLIED STEM CELL TECHNOLOGIES Locsense

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- Gut mimic: *in vitro* translation
- Impedance spectroscopy & transepithelial electrical resistance (TEER)
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- Measuring the tight junction/barrier
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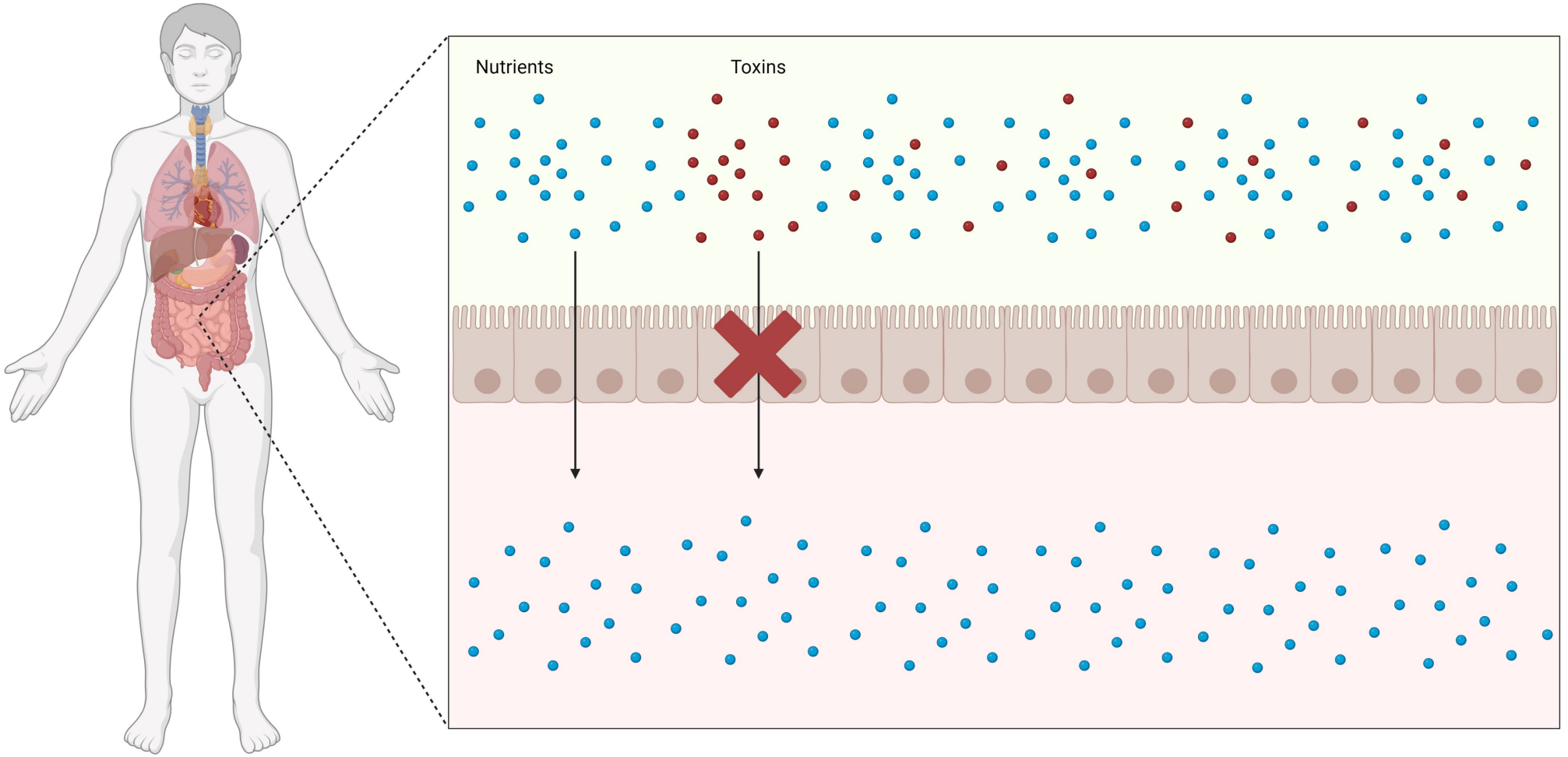
Function of the gut



Impaired gut barrier leads to:

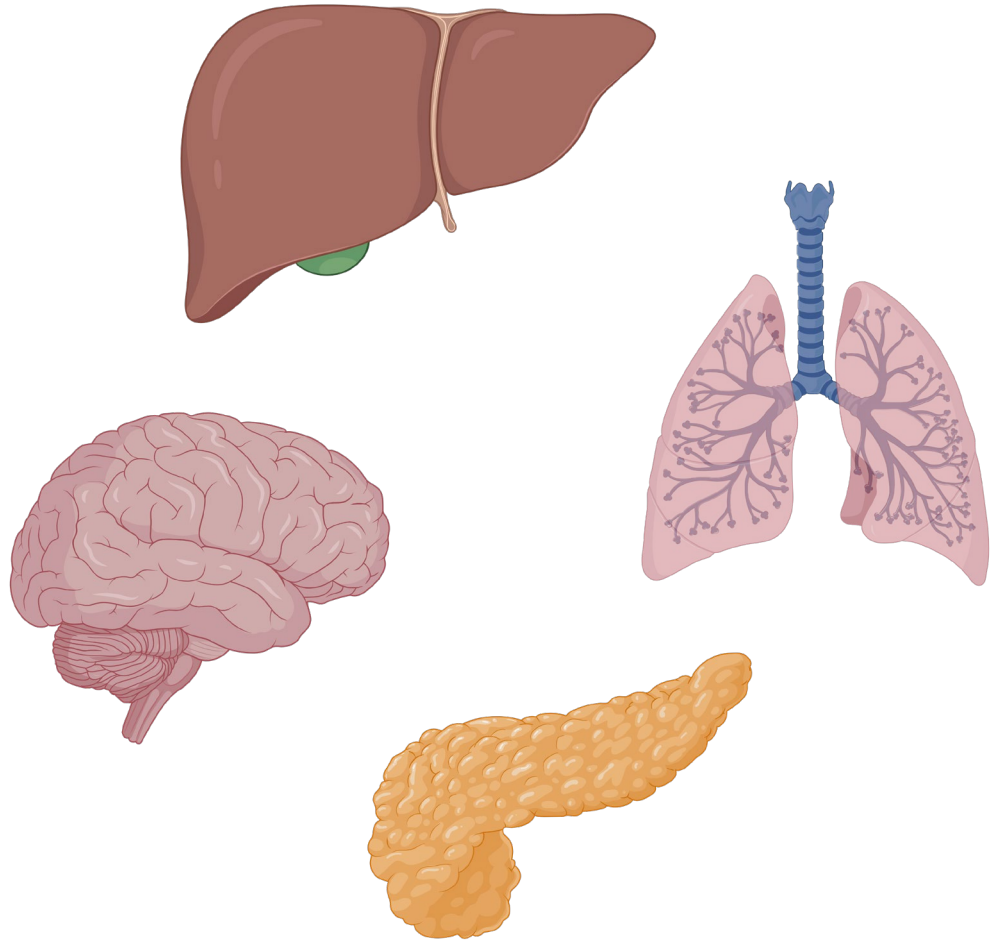
Liver disease [1]

Brain damage [2]

Lung disease [3]

Pancreas [4]

Etc.....



[1] A. Albillos et al. “The gut-liver axis in liver disease: Pathophysiological basis for therapy”, *J. Hepatol* 2020

[2] J. Appleton et al. “The Gut-Brain Axis: Influence of Microbiota on Mood and Mental Health,” *Integr Med* 2018

[3] L. Qu et al. “COPD and Gut–Lung Axis: How Microbiota and Host Inflammasome Influence COPD and Related Therapeutics,” *Front Microbiol* 2022

[4] Z. Zhang, et al. “Intestinal homeostasis and inflammation: Gut microbiota at the crossroads of pancreas-intestinal barrier axis,” *Eur J Immunol* 2022

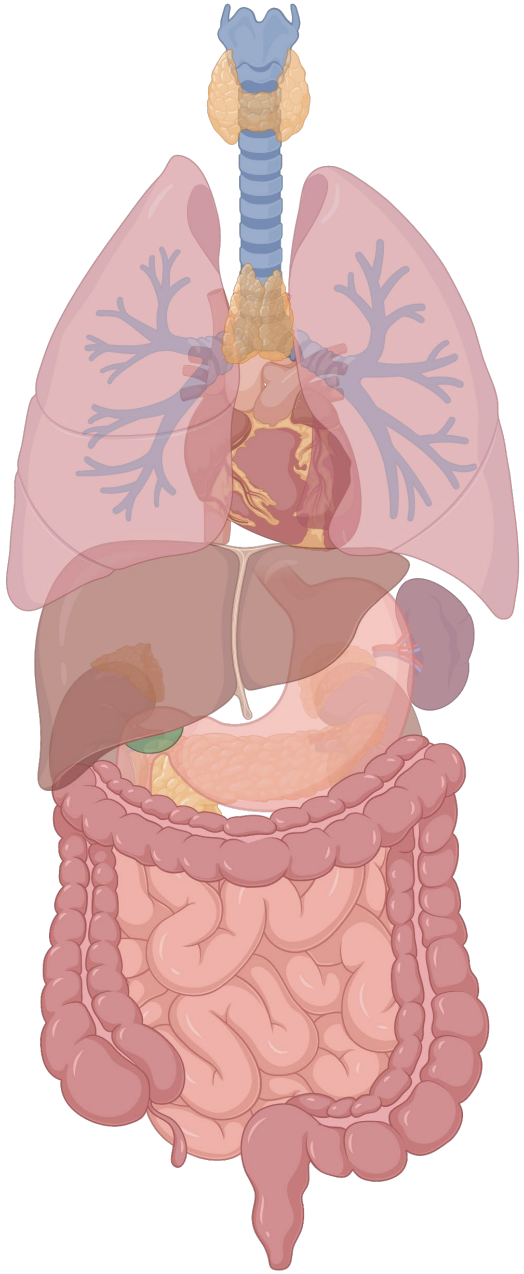
In vitro analysis

A representable *in vitro* gut mimic is necessary to investigate:

- which factors influence the barrier function
- the cross talk between the gut and other organs

Aim:

- Propose a representable gut mimic
- Evaluate the barrier function of the gut mimic



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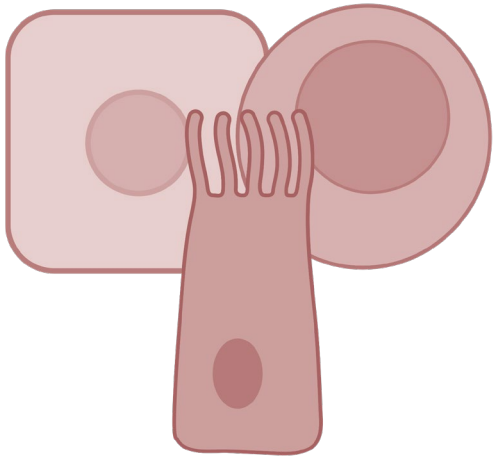
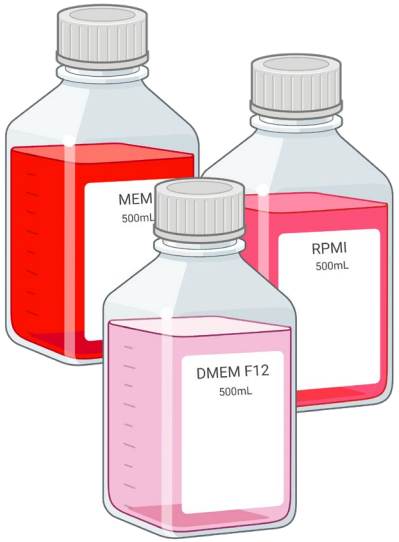
In vitro translation of the gut barrier

1. Cell type

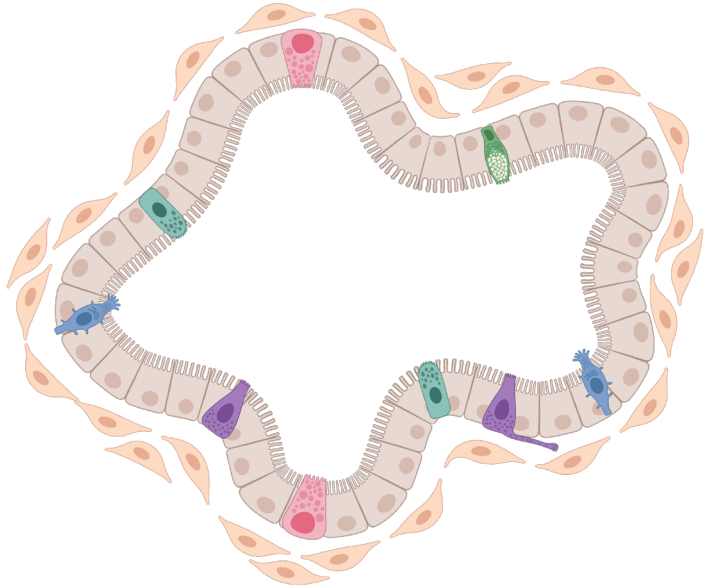


2. Cell medium



3. 2D/3D structure

Cell type [1, 2]

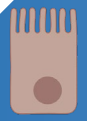


- Villi – differentiated compartment
  - Enterocytes – nutrient absorption
  - Goblet cells – production of mucus
  - Enteroendocrine cells – hormone production
  - Cup cells – function not clear
- Crypts – proliferative compartment
  - Paneth cells – regulate gut microbiota
- Other
  - Microfold cells (M-cells) – contact with immune system
  - Tuft cells – contact with immune system and taste perception

[1] V. Bonis, et al. “The Intestinal Epithelium – Fluid Fate and Rigid Structure From Crypt Bottom to Villus Tip,” *Front. Cell Dev. Biol.* 2021
[2] F. Gerbe, et al. “The intestinal epithelium tuft cells: specification and function” *Cell Mol. Life Sci.* 2012

Cell type

Few - simple

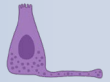


Enterocyte

Complex - many

Enterocyte

Goblet cell



Enteroendocrine cell

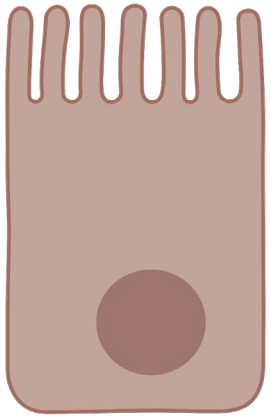
Cup cell

Paneth cell

M-cell

Tuft cell

Cell type



Caco-2 [1]
Enterocyte

HT29-mtx [1]
Goblet-like cell

Derived from the colon

ATCC® HTB-37™

20% FBS

90% cell number [1]

Derived from the colon

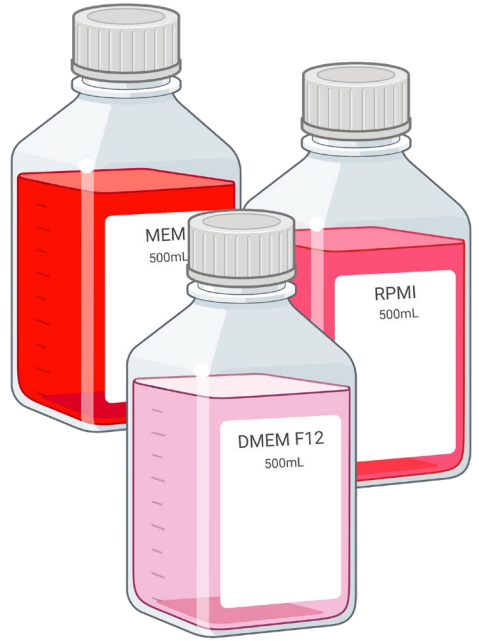
Mucus producing cells

10% FBS

10% cell number [1]

[1] P. Hoffmann et al., "Caco-2/HT29-MTX co-cultured cells as a model for studying physiological properties and toxin-induced effects on intestinal cells," *PLoS One* 2021

Culturing medium



Cultured in DMEM-F12-GlutaMAX

- Alternative DMEM-F12-Glutamine (ATCC® 30-2006™)

1% Supplemented with non-essential amino acids

1% Pen/strep, removed during standard culture and monolayer formation

- Penicillin-Streptomycin Solution (ATCC® 30-2300™)

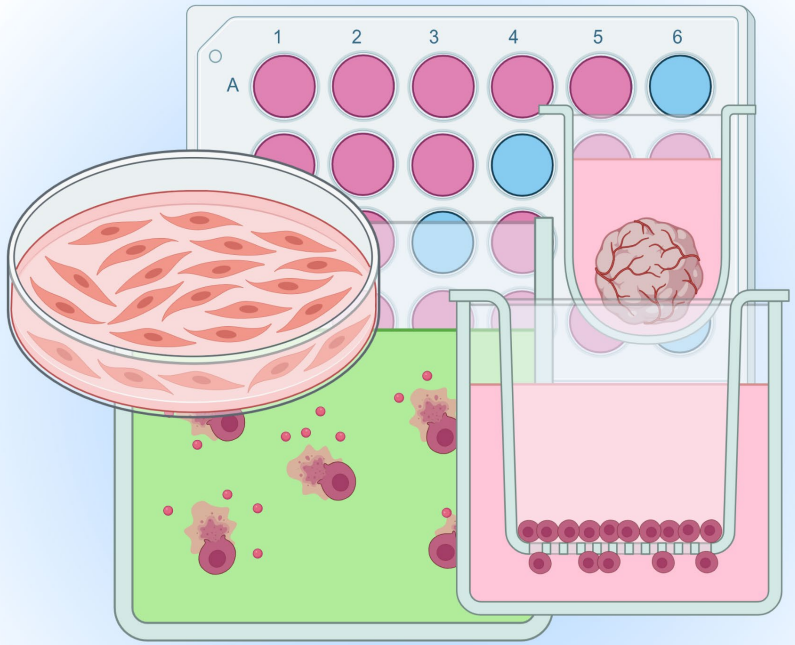
15% FBS, compromise

- Fetal Bovine Serum (FBS) (ATCC® 30-2020™)

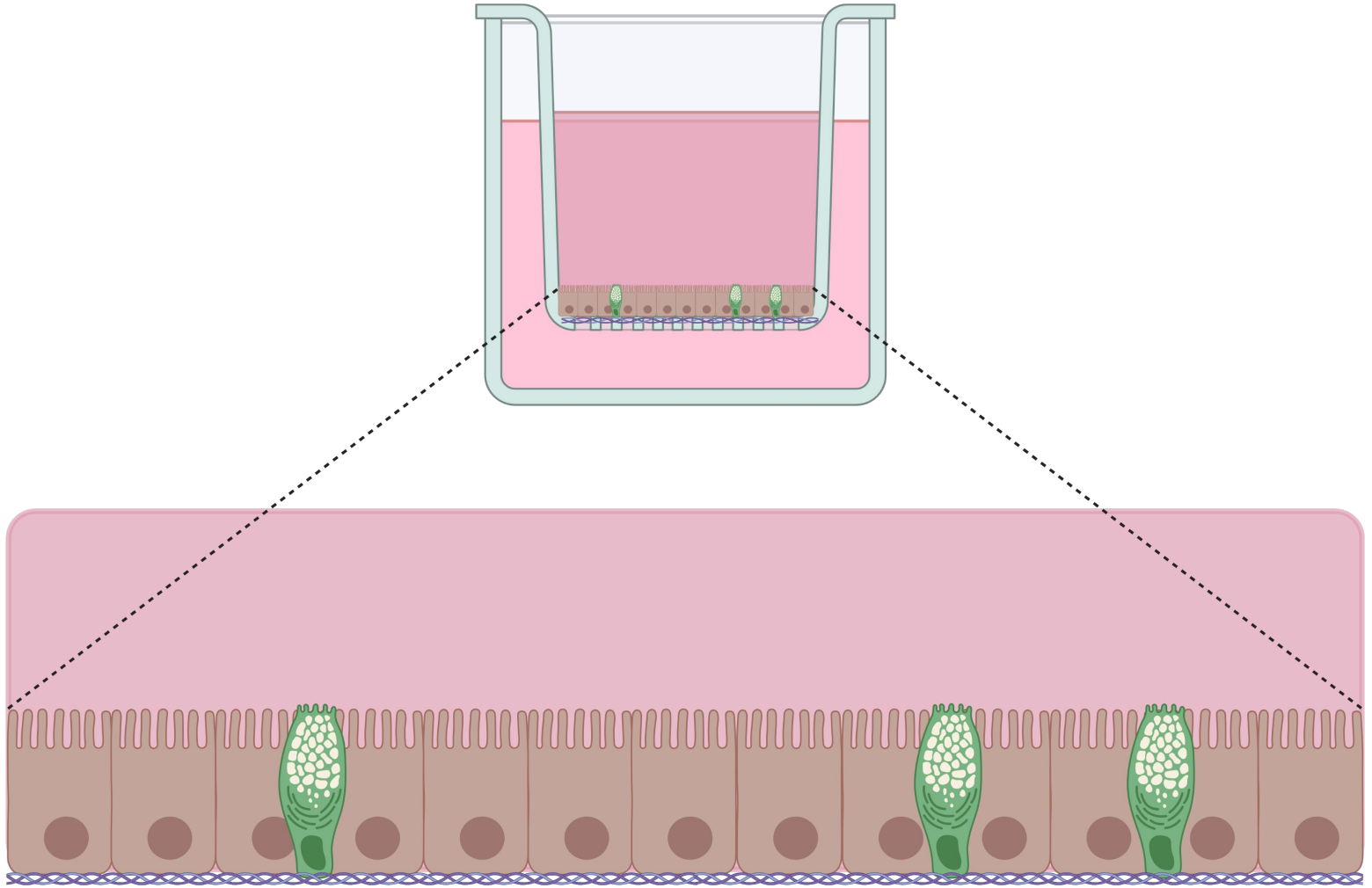
Passaged via trypsin-EDTA

- Trypsin-EDTA (ATCC® 30-2101™)

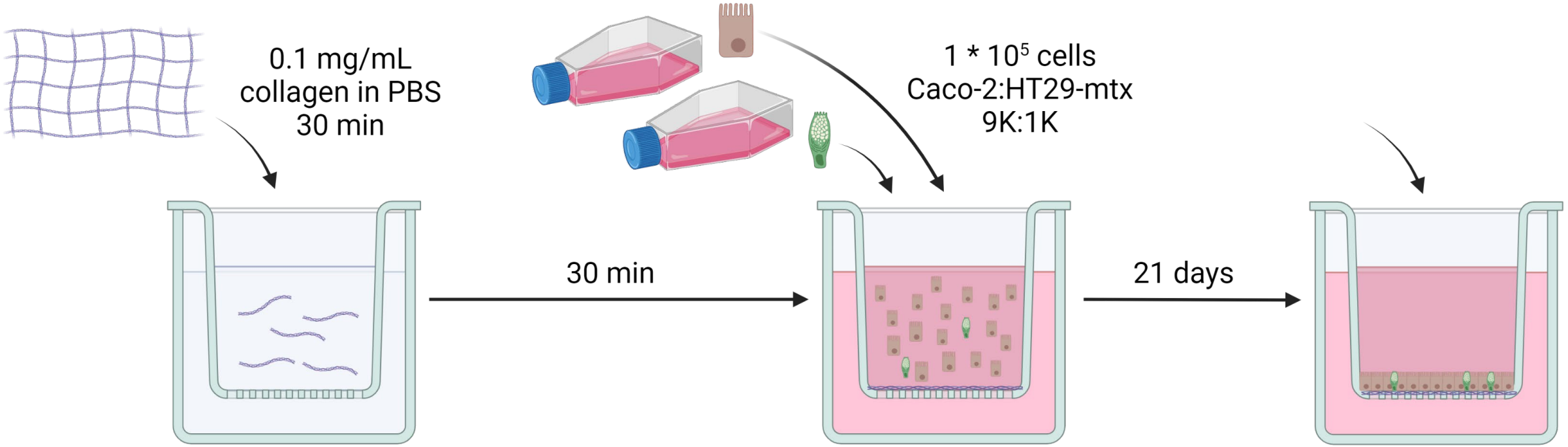
3D structure



Transwell



Complete culturing procedure



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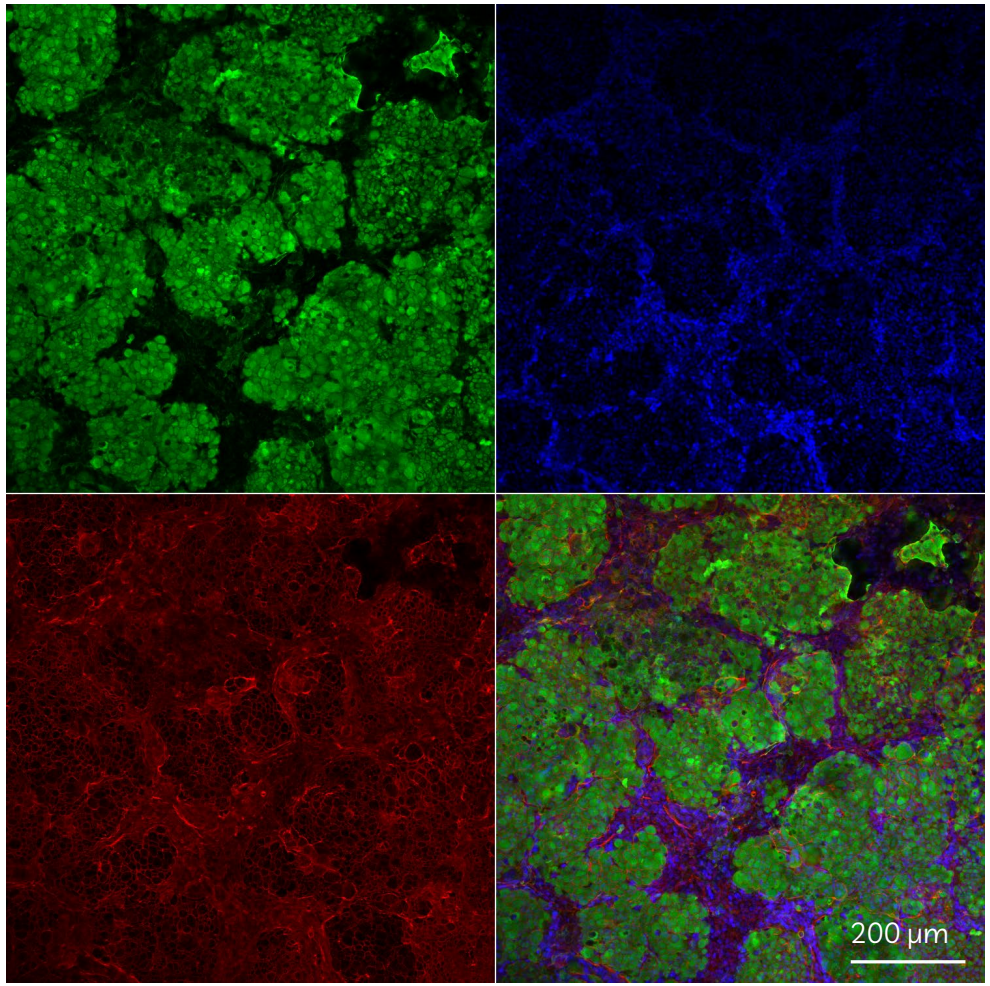
Caco-2, HT29-mtx co-culture



Green = Wheat germ agglutinin (WGA) → mucus marker

Blue = DAPI → nucleus marker

Red = Actin → cytoskeleton marker

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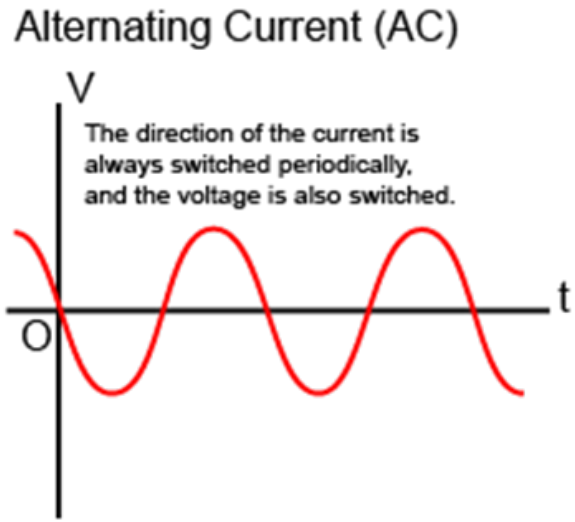
Impedance spectroscopy and Transepithelial electrical resistance (TEER)

Impedance = The combined effect of **resistance** and **reactance** in a circuit [1]

All the obstacles that impede the current to flow to the other electrode

TEER = The measurement of **electrical resistance** across a cellular layer [1]

Tight junction resistance between the cells = barrier function



[1] Srinivasan, B. et al. "TEER measurement techniques for in vitro barrier model systems" *J. Lab. Autom.* 2015

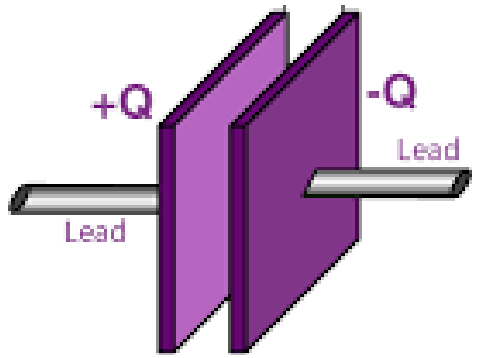
Impedances = Resistance + Reactance

Resistance

Constant impedance of electrical current

Reactance - capacitance

Capability to store and release electrical charge



Resistance and capacitance

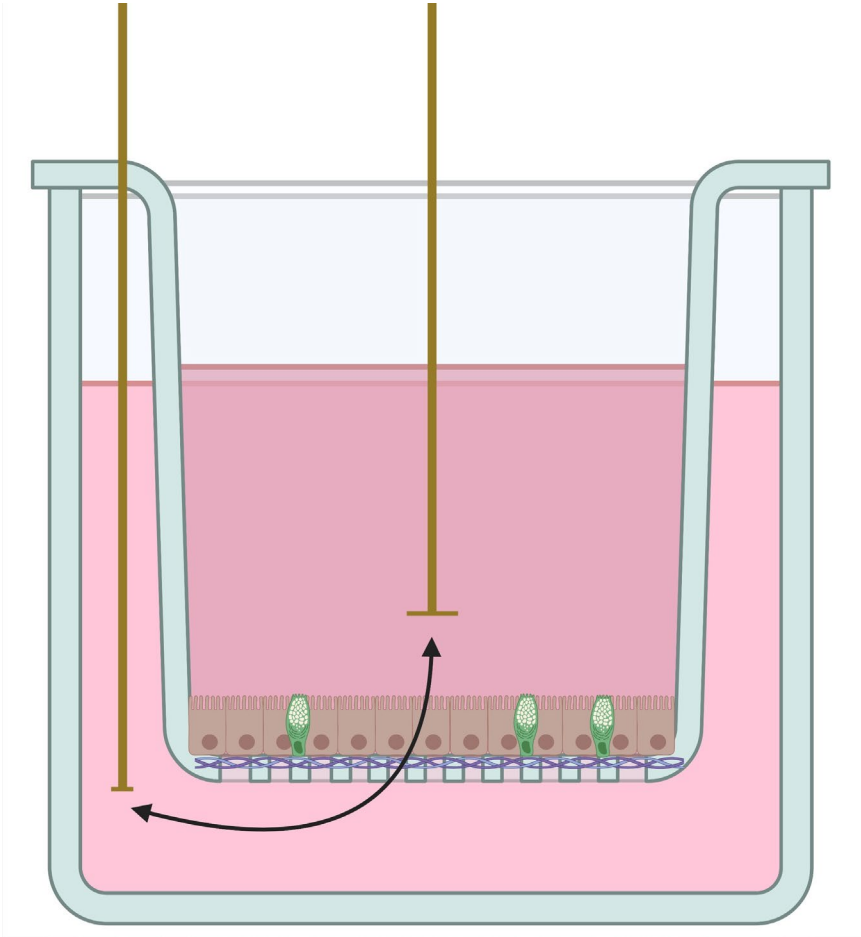
Resistance = swimming

It takes longer to get to the other side, but going is continuous

Capacitance = a ferry boat

It has to wait for other passengers, but it travels fast when it is going

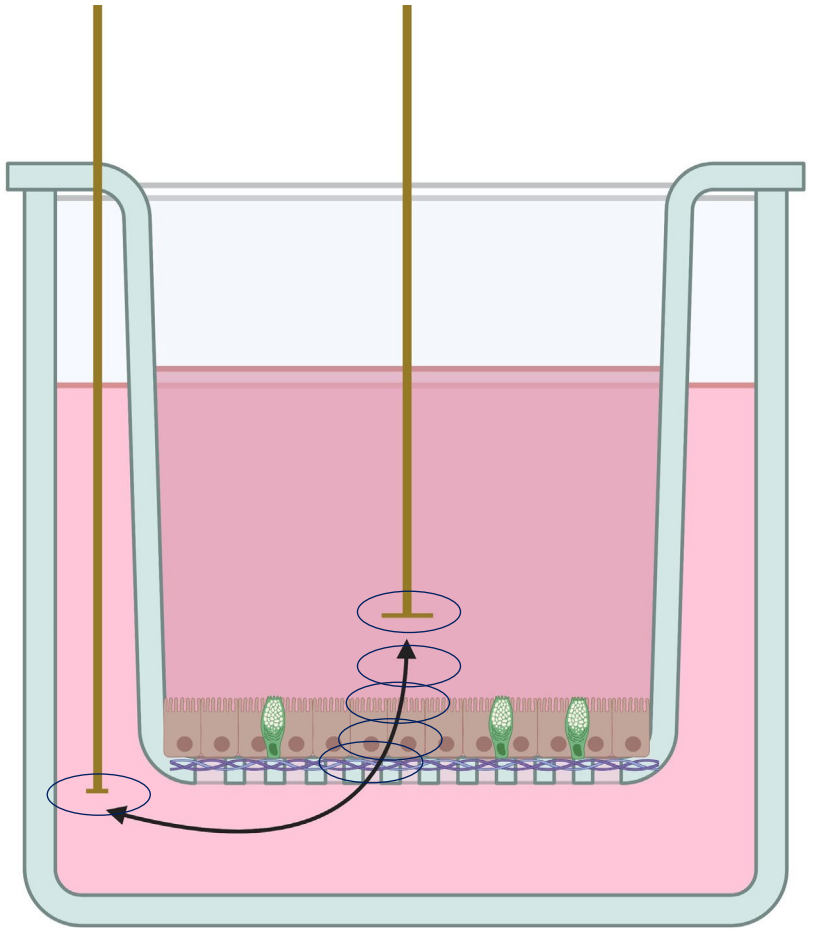
Impedance measurement



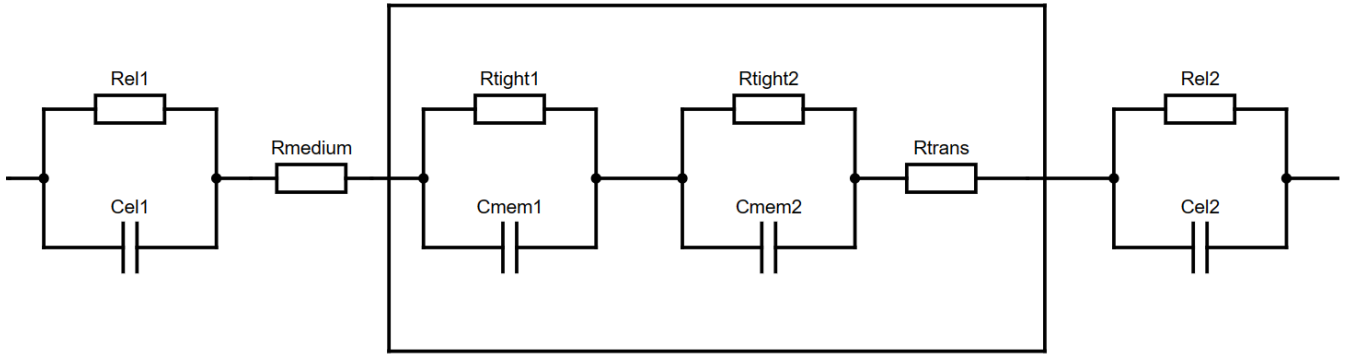
Circuit model fitting

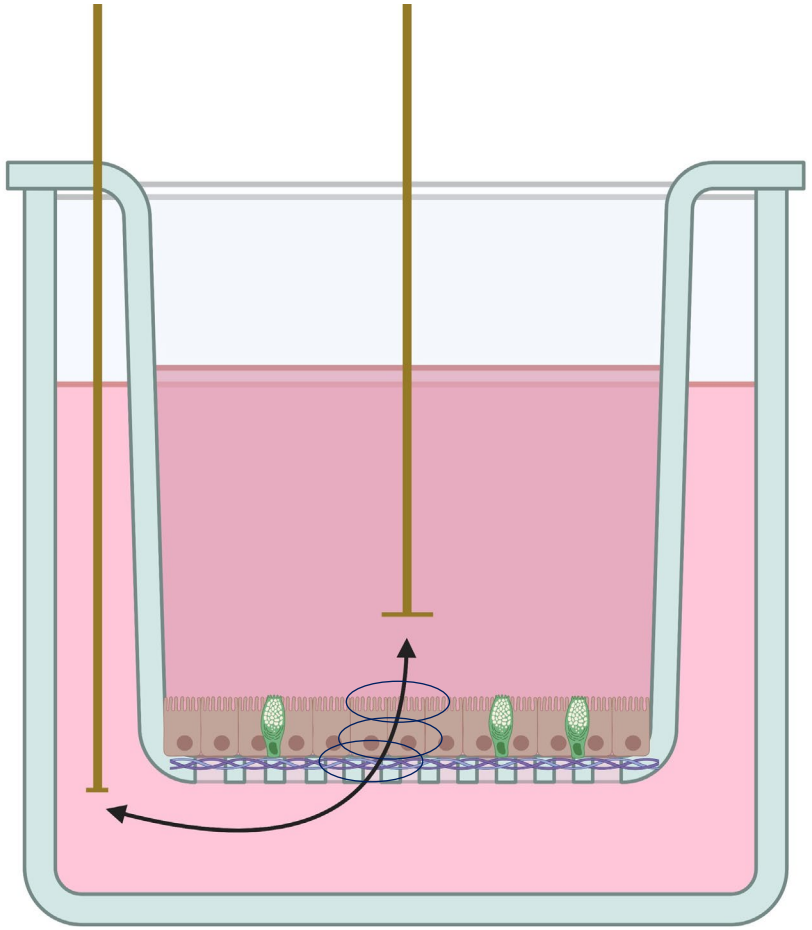
Components of the circuit:

1. R_{el1} + C_{el1} = Resistance + capacitance of electrode 1
2. R_{medium} = Resistance of the medium
3. R_{tight1}+C_{mem1} = the tight junction resistance and membrane capacitance of the apical side
4. R_{tight2}+C_{mem2} = the tight junction resistance and membrane capacitance of the basolateral side
5. R_{trans} = resistance of the coated transwell
6. R_{el2} + C_{el2} = Resistance + capacitance of electrode 2



Transepithelial electrical resistance (TEER)





The cells influence

R_{tight1}+C_{mem1} = the tight junction resistance and membrane capacitance of the apical side

R_{tight2}+C_{mem2} = the tight junction resistance and membrane capacitance of the basolateral side

R_{trans} = resistance of the coated transwell

The total TEER = R_{tight1}+ R_{tight2}

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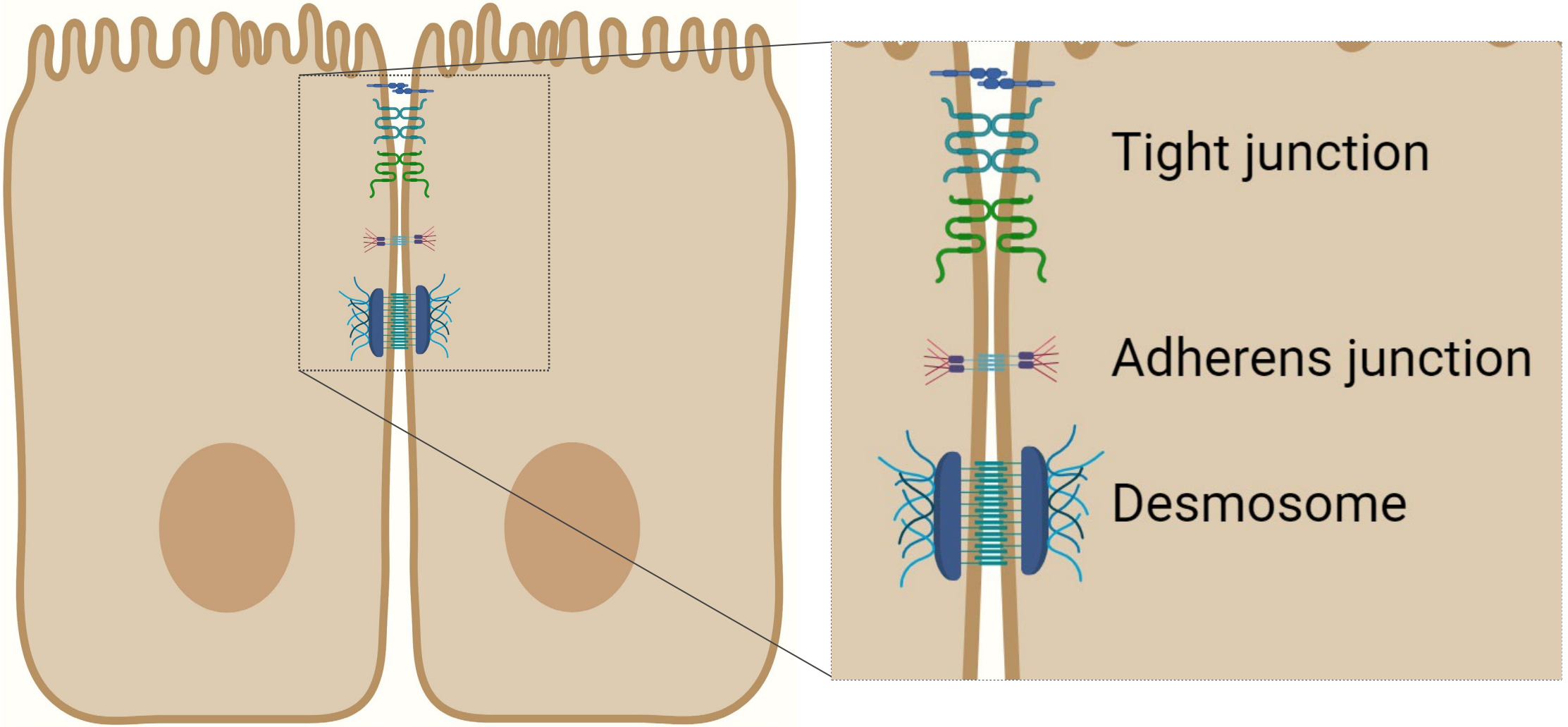
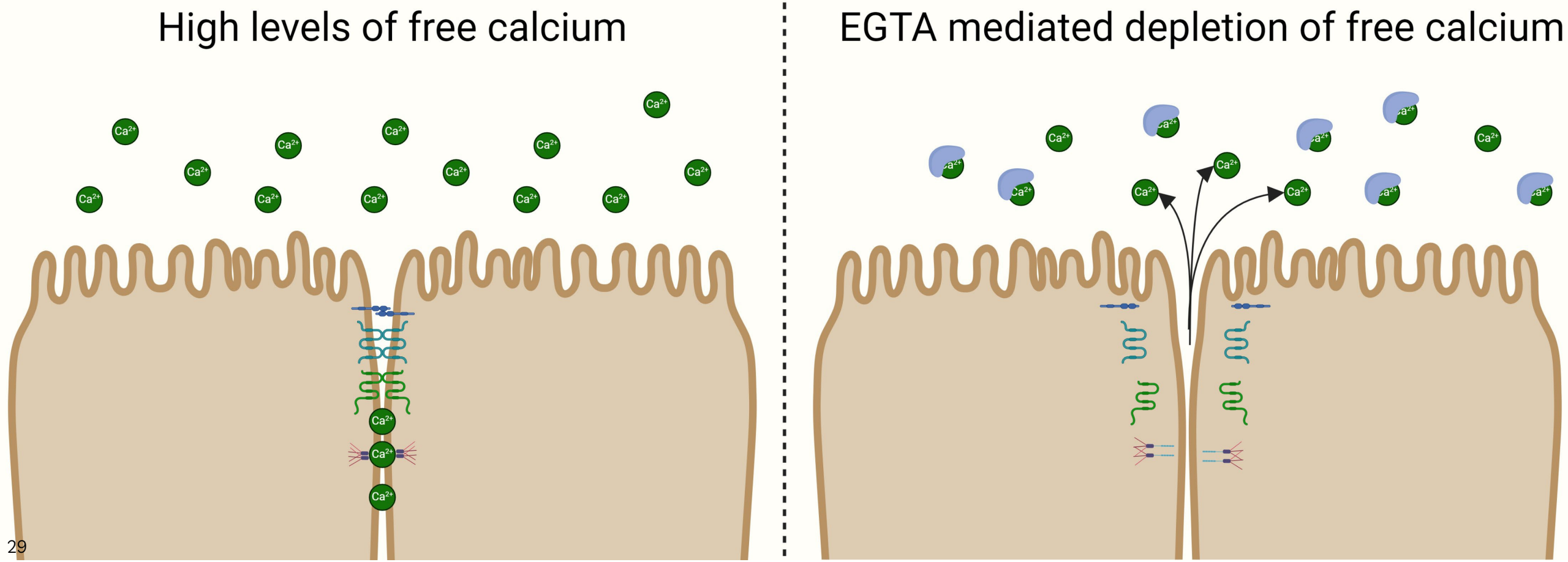
Tight junctions



EGTA and tight junctions

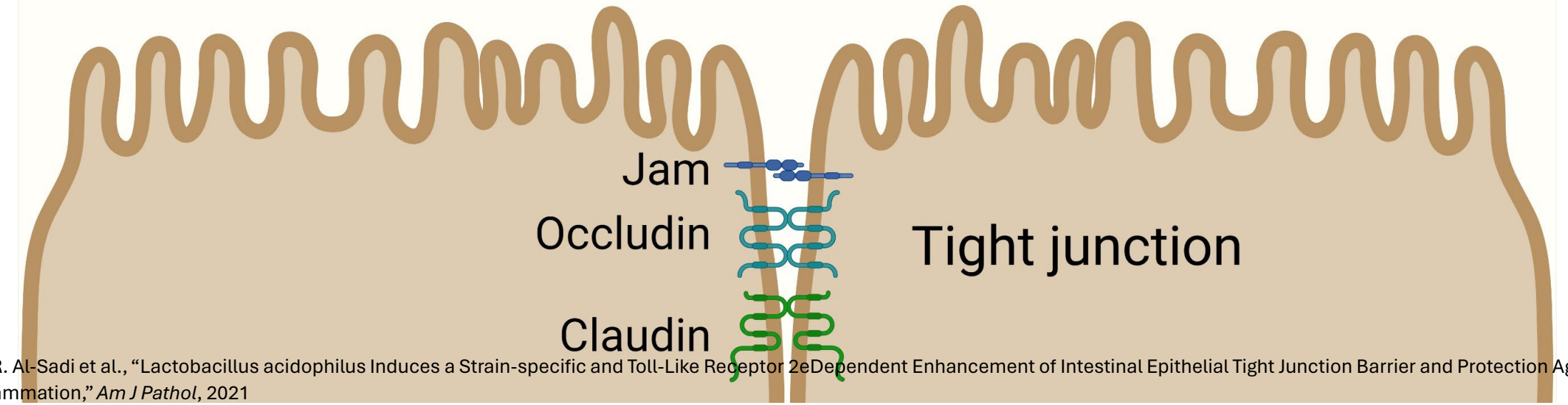


Lactobacillus acidophilus (LA) and tight junctions [1]

LA attaches to the apical side of the Enterocyte → ↑ TLR-2 membrane protein

TLR-2 membrane protein → ↑ interaction with TLR-1 and TLR-6

TLR2/TLR1 and TLR2/TLR6 → ↑ occludin



[1] R. Al-Sadi et al., "Lactobacillus acidophilus Induces a Strain-specific and Toll-Like Receptor 2eDependent Enhancement of Intestinal Epithelial Tight Junction Barrier and Protection Against Intestinal Inflammation," *Am J Pathol*, 2021

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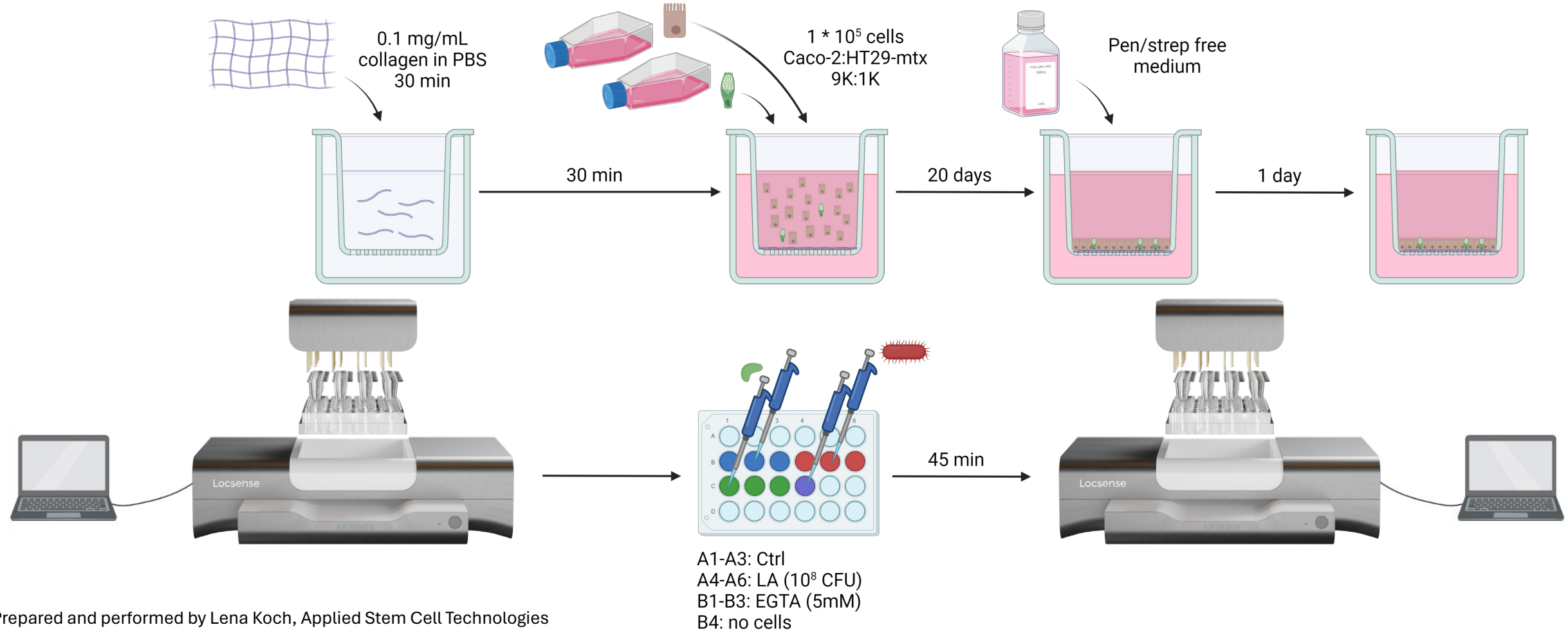
Complete procedure



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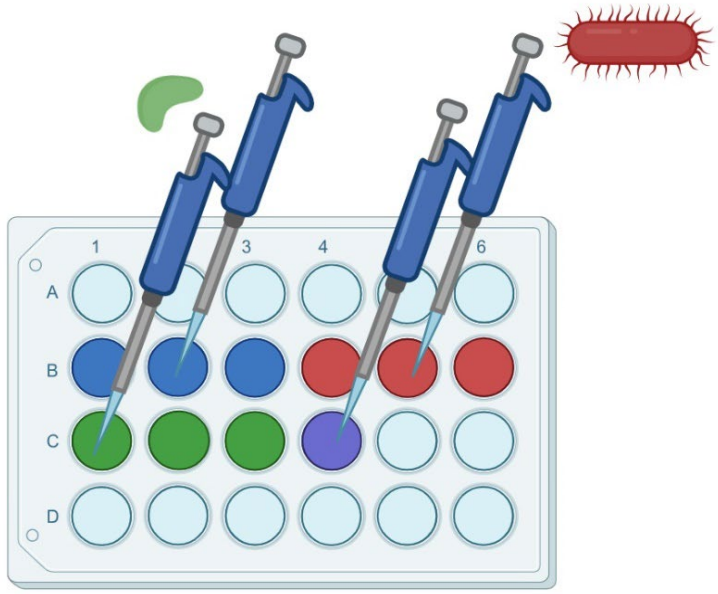
Conditions



A1-A3: Ctrl
A4-A6: LA (10^8 CFU)
B1-B3: EGTA (5mM)
B4: no cells

T=0 → Measurement 1

After measurement → LA is added to LA wells

T=24 → Measurement is performed

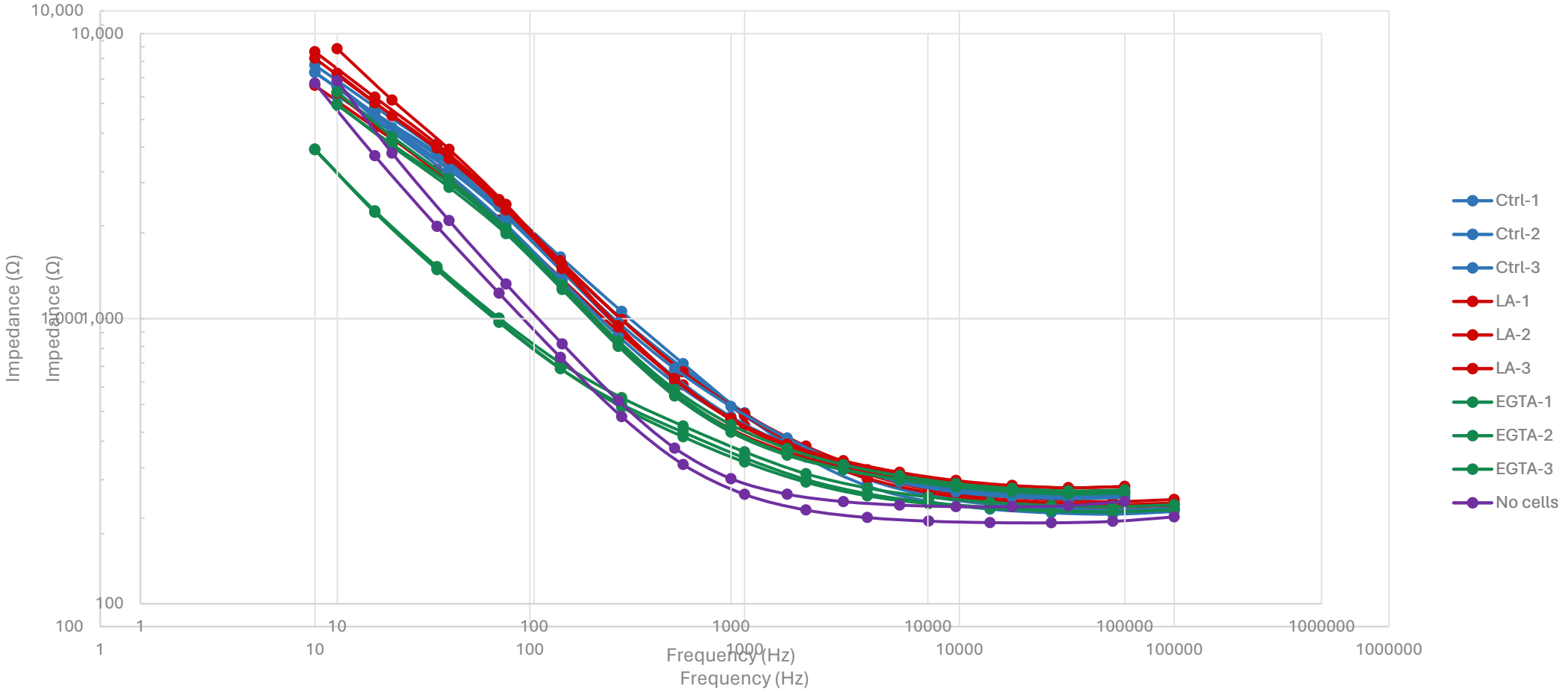
After T=24 → medium of EGTA wells is removed, EGTA in PBS is added for 45 min → old medium (+10% fresh) is added

T=25 → Measurement is performed

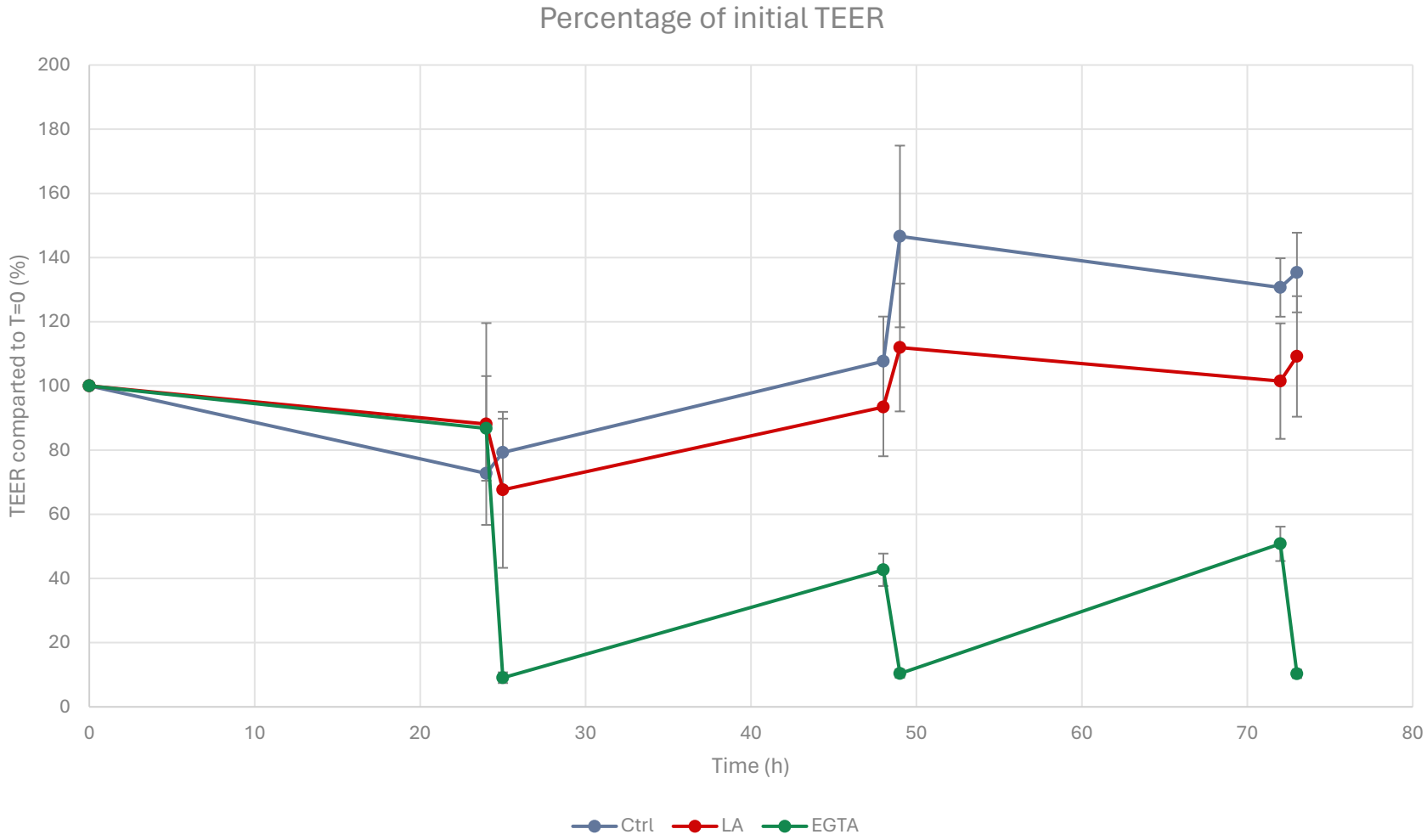
Repeated at T=48 + T=49 and T=72 + T=73

Prepared and performed by Lena Koch, Applied Stem Cell Technologies

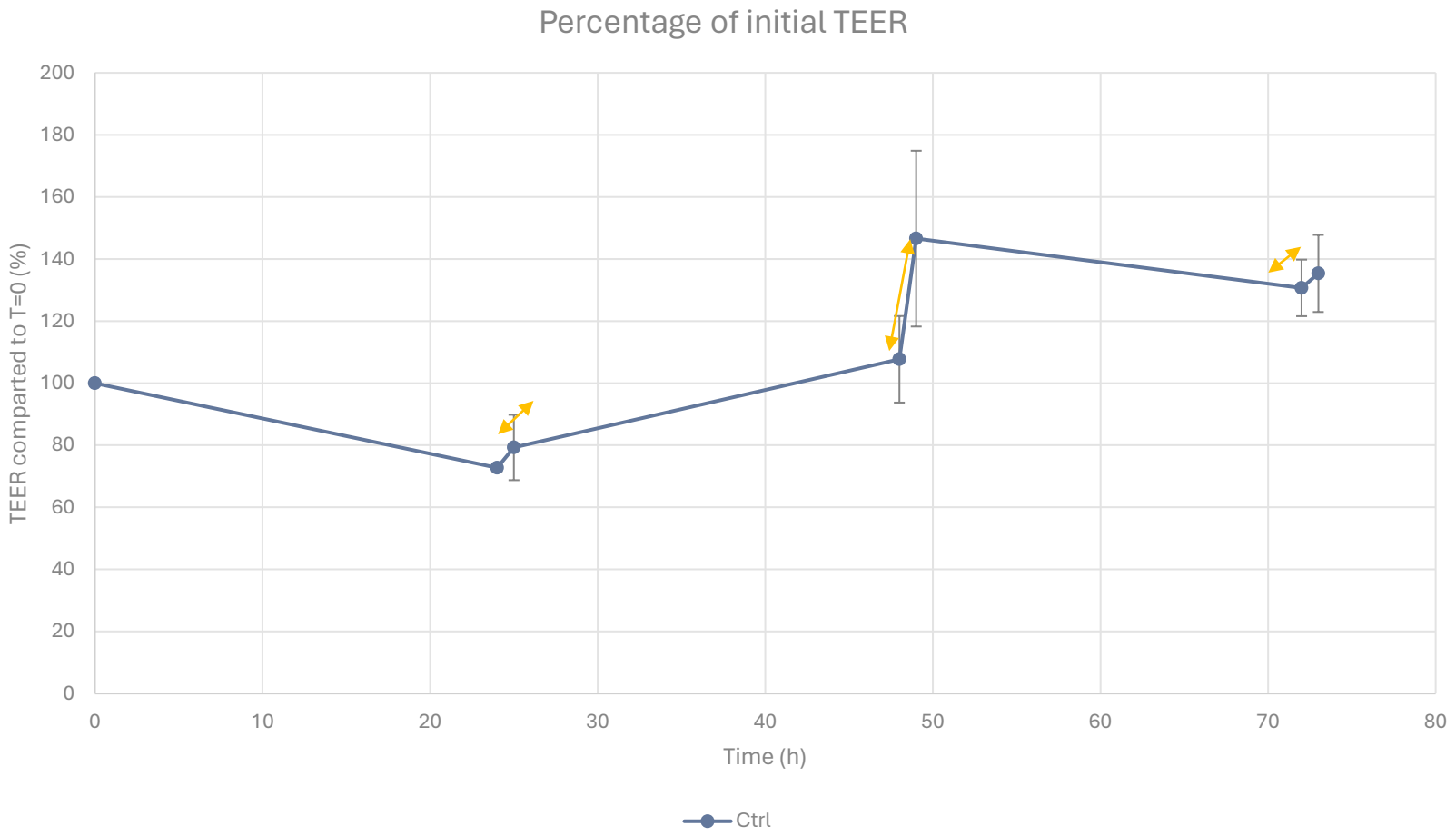
Raw impedance data



TEER results



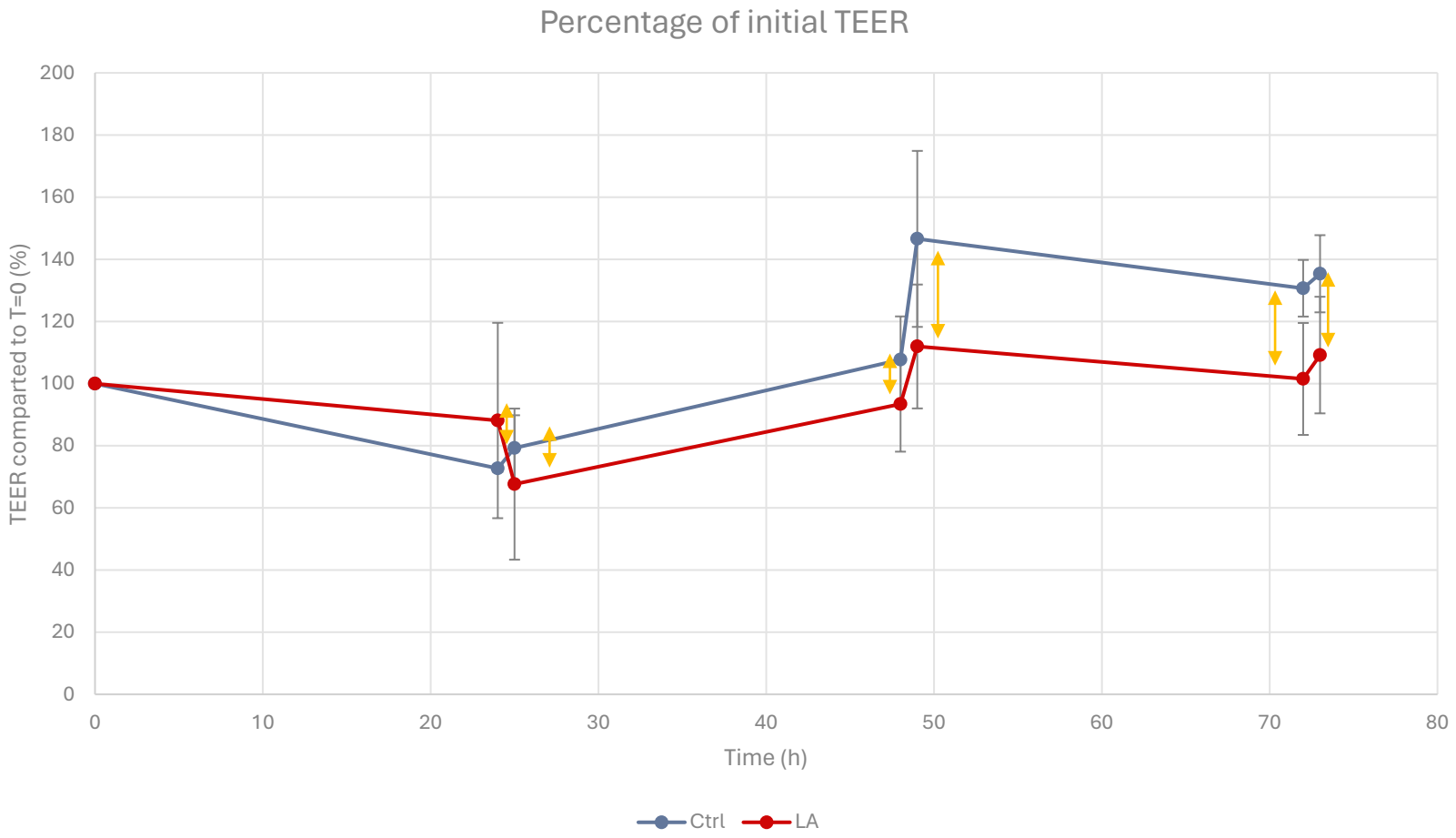
Temp. does not significantly change TEER



Difference between 2 measurements

	Ctrl
Day 1	6,5
	ns
Day 2	38,9
	ns
Day 3	4,7
	ns

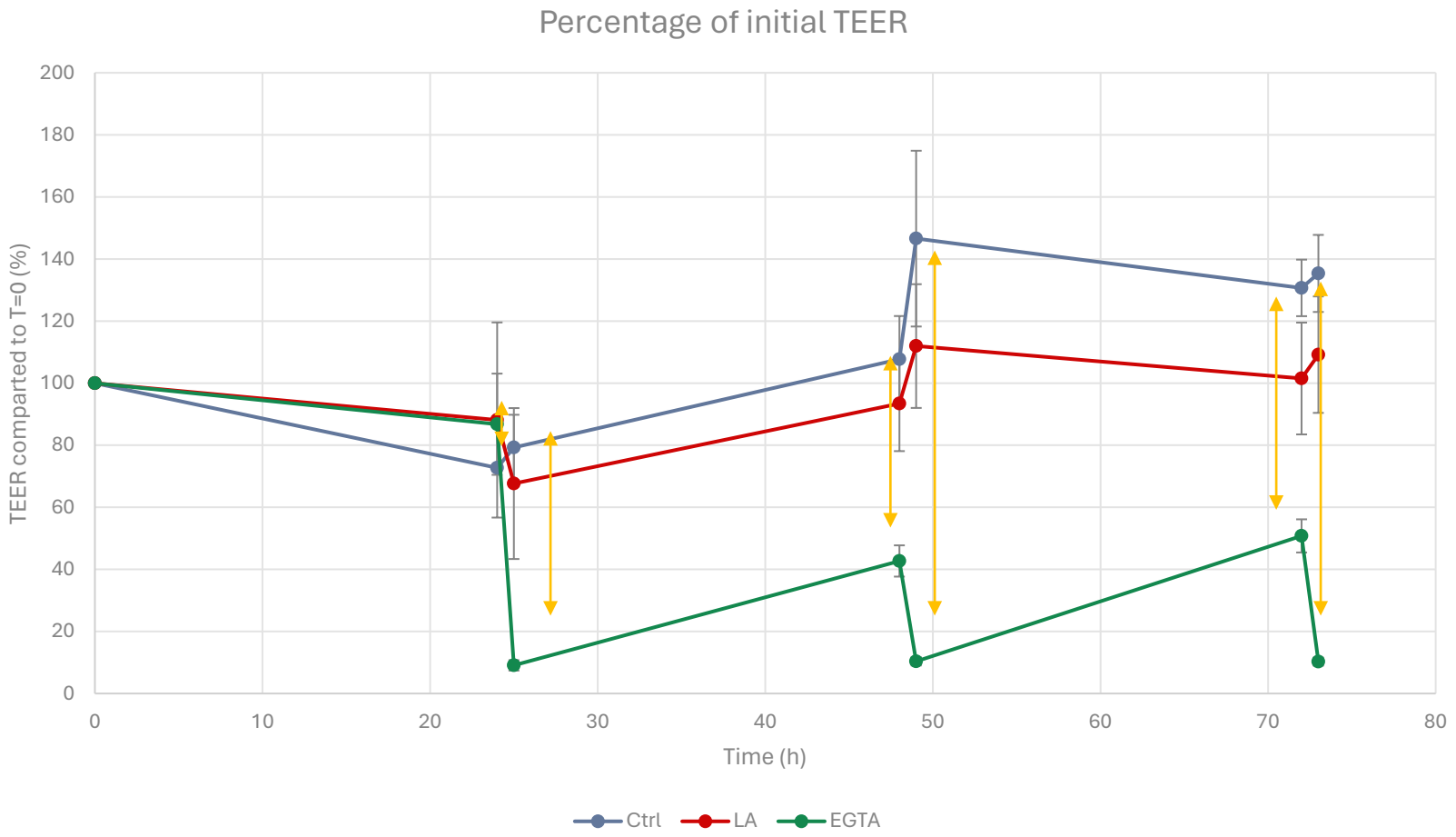
LA does not significantly change the TEER



Difference between Ctrl and LA

Time (h)	LA	
24	15,4	ns
25	-11,6	ns
48	-14,3	ns
49	-34,6	ns
72	-29,2	ns
73	-26,2	ns

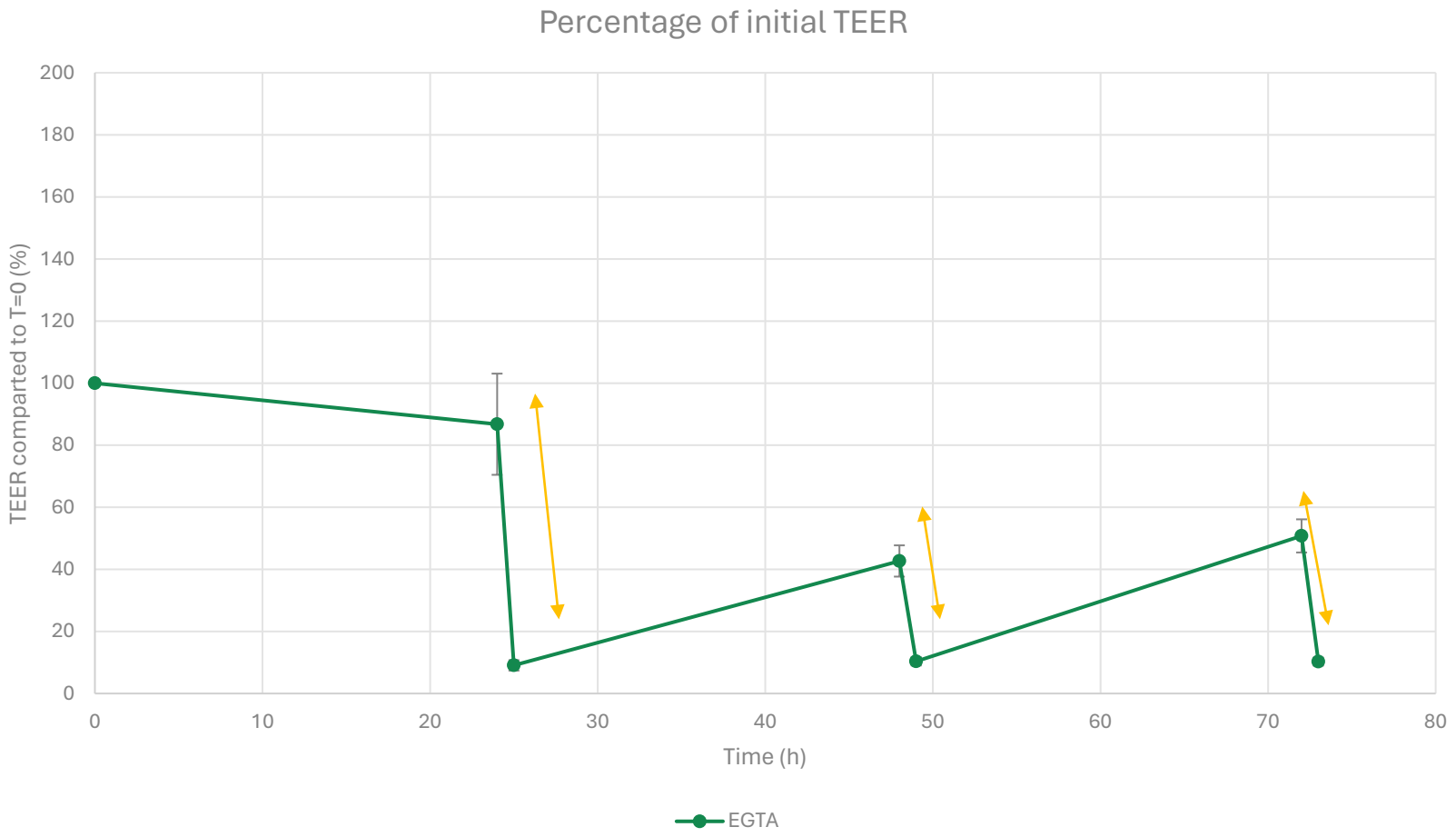
EGTA does significantly change the TEER



Difference between Ctrl and LA or EGTA

Time (h)	LA		EGTA	
24	15,4	ns	14,0	ns
25	-11,6	ns	-70,2	***
48	-14,3	ns	-65,0	**
49	-34,6	ns	-136,2	**
72	-29,2	ns	-79,9	***
73	-26,2	ns	-125,1	****

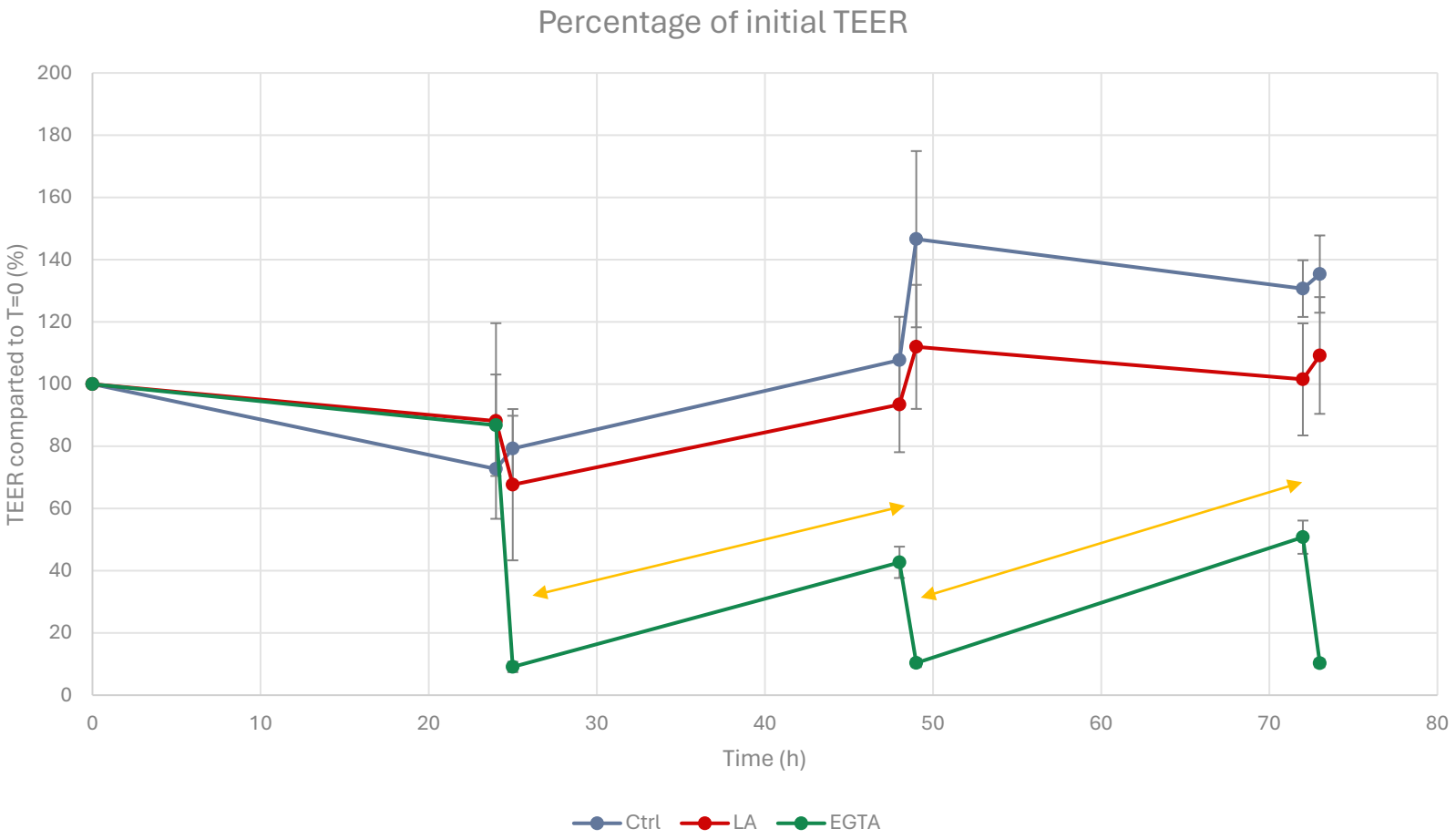
EGTA does significantly change TEER in 45 min



Difference between before and after EGTA addition

	EGTA
Day 1	-77,7
	***
Day 2	-32,3
	***
Day 3	-40,5
	***

EGTA treatment is reversible



EGTA is capable of partial recovery after treatment

	Difference	
25-48	33,6	**
49-72	40,4	**

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Conclusions cell culturing

- A Caco-2 (ATCC® HTB-37™) HT29-mtx co-culture is a representable *in vitro* gut mimic
- 9:1 ratio
- Cultured in DMEM-F12-GlutaMAX culturing medium
    - 15% FBS, compromise
- 21-day pre-culture

Conclusions impedance spectroscopy

- Impedance spectroscopy is a non-destructive measurement technique
- Circuit model fitting enables to extract TEER data from complex impedance measurements
- The Artemis device
  - Automated measurement
  - User friendly
  - Time saving
  - Reduces cross contamination risks

Conclusions EGTA and LA

- Temperature influences, but changes the TEER non-significantly
- Lactobacillus acidophilus does not significantly influence the tight junction barrier
- EGTA successfully hampers the tight junctions
- EGTA barrier function impairment is reversible

Collaboration

This webinar was a collaboration between Locsense B.V. and Applied Stem Cell Technologies, University of Twente, the Netherlands

Work is conceptualized and performed by Lena Koch

Conceptualization and funding acquisition Kerensa Broersen

Artemis TEER/impedance spectroscopy instrument is provided by Locsense

THANKS

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Questions?

For more information:

www.atcc.org/tox

https://locsense.nl