Butyric acid and derivatives: *In vitro* effects on barrier integrity of porcine intestinal epithelial cells quantified by transepithelial electrical resistance

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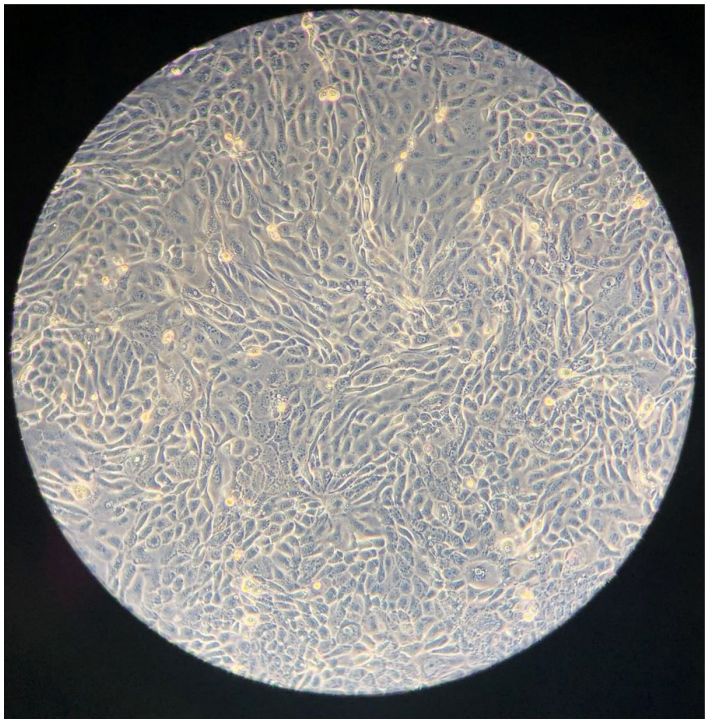
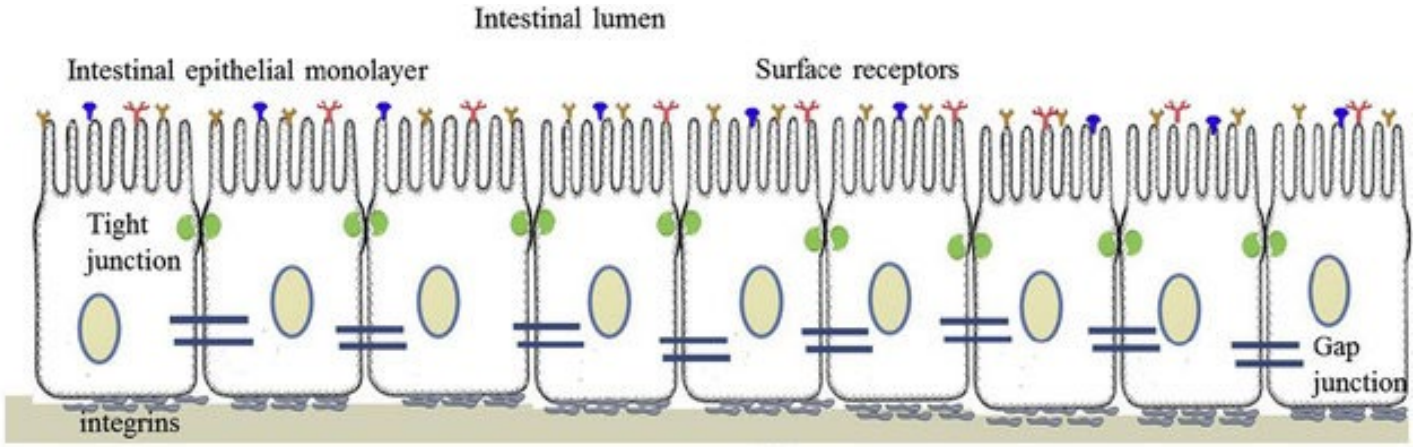
Background: IPEC-J2

- Intestinal porcine epithelial cells (IPEC)-J2
  - Immortal cell line
  - Derived from unsuckled neonate piglet jejunal intestine (Berschneider, 1989)



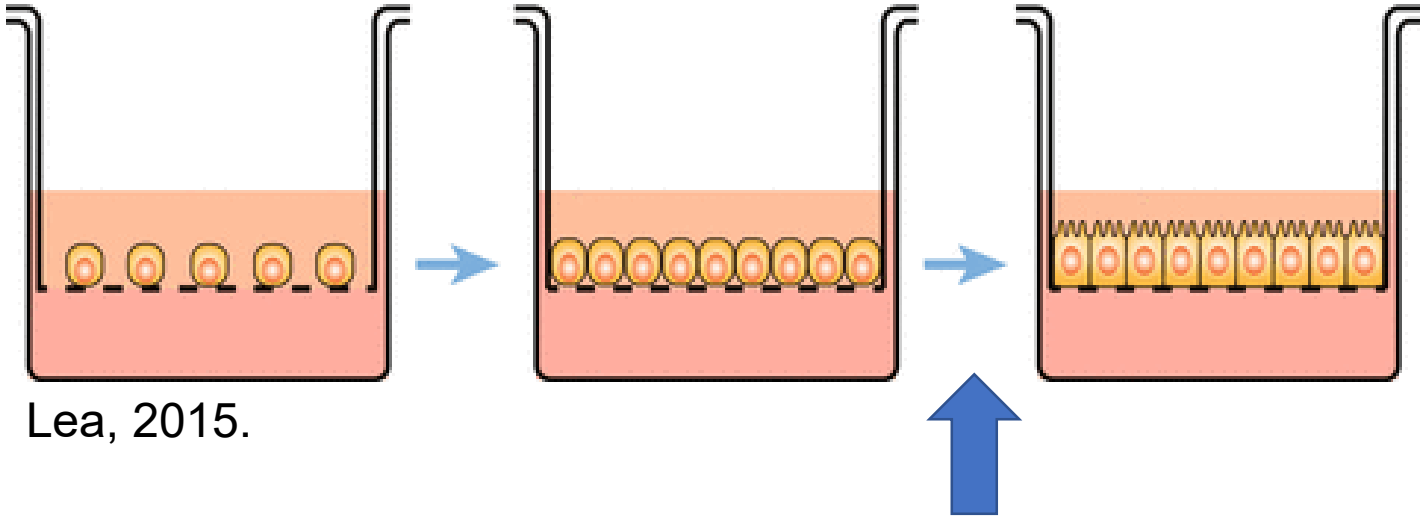
Barrier Integrity of the Intestine

- Intestinal epithelial cells form a monolayer
- Barrier function is semipermeable and vital to nutrient absorption, water retention, and preventing pathogen invasion



Gokulan et al., 2017

Intestinal epithelial cell monolayers *in vitro*

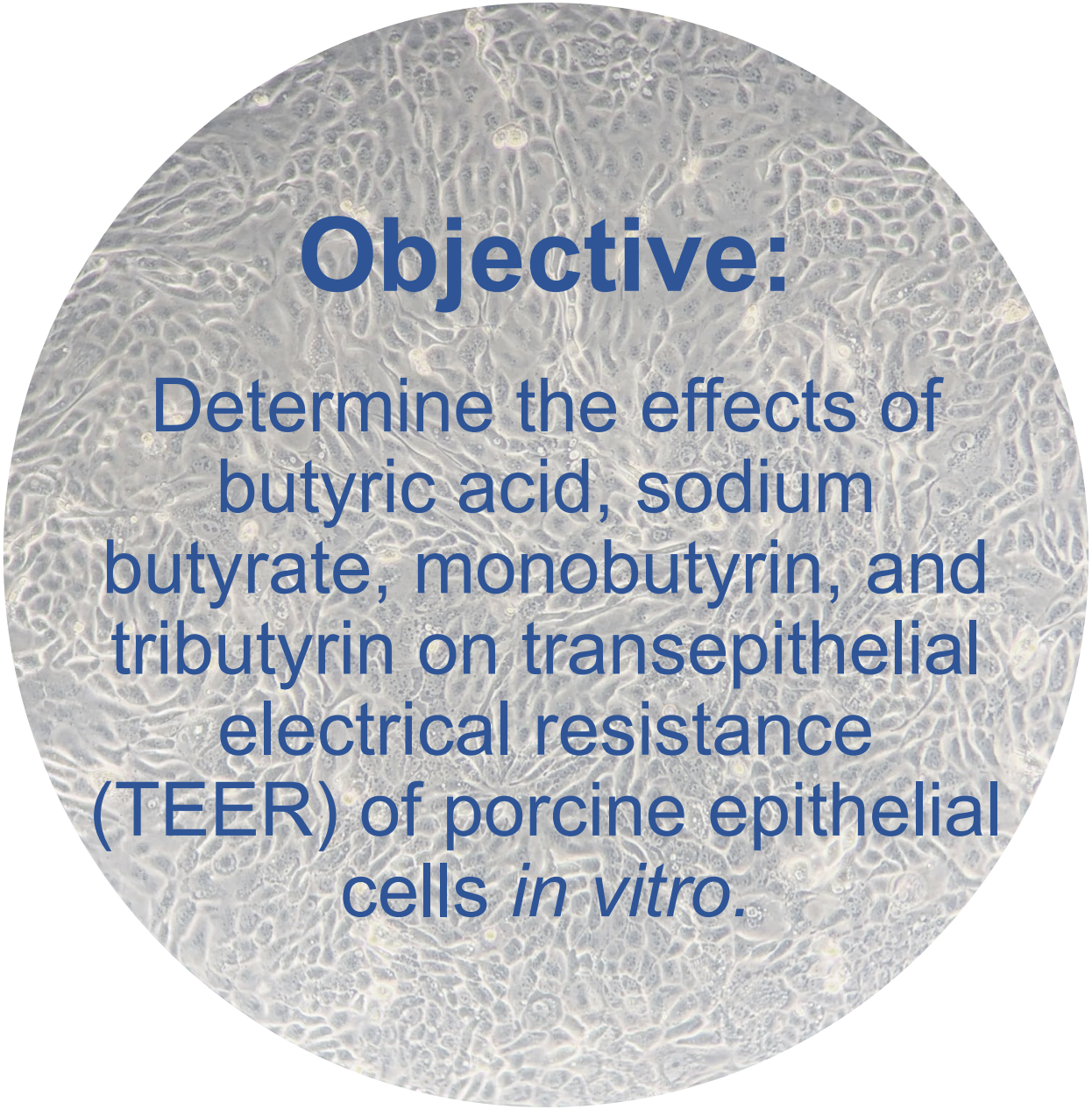


Lea, 2015.

Differentiation:
Expression of tight junction proteins and polarized across apical and basal sides

Butyric acid and derivatives

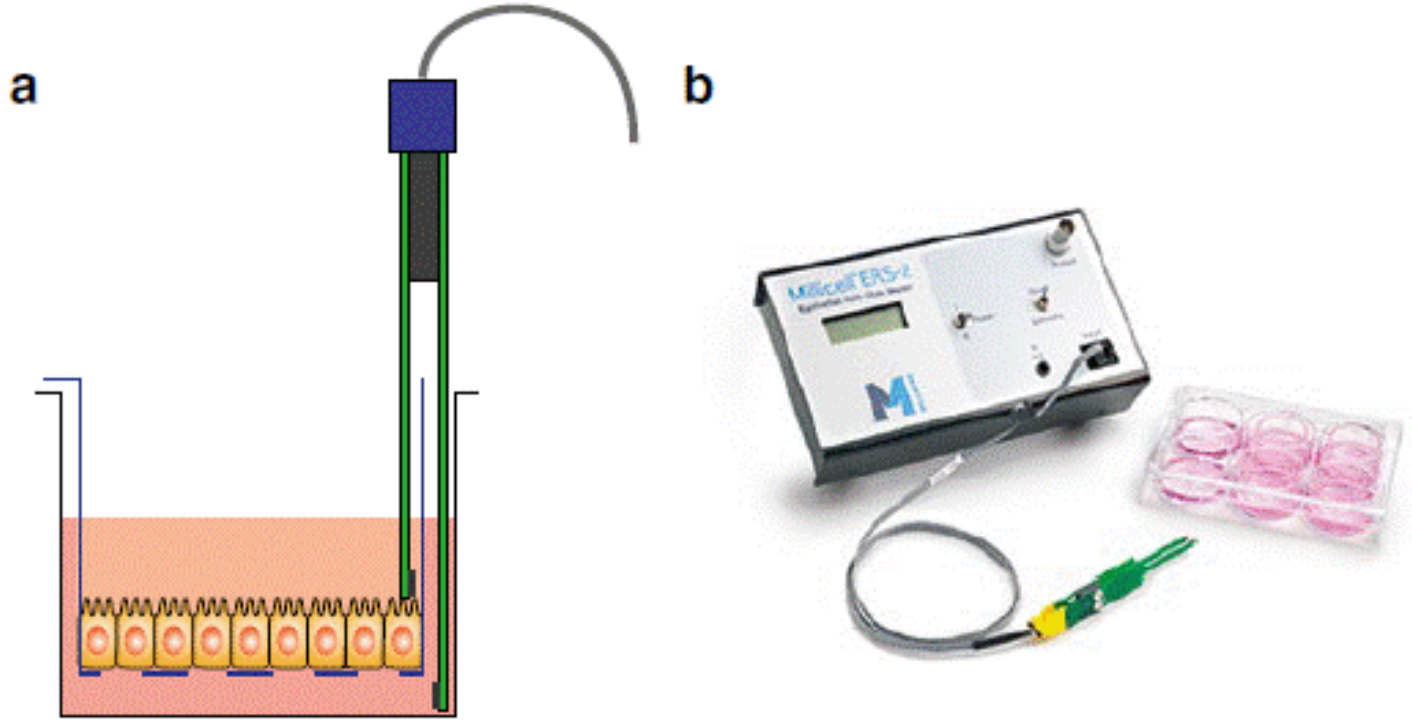
- Butyrate can be used as an energy source for enterocytes.
- *In vitro*
  - Butyrate and derivatives increase TEER in human and porcine intestinal cells (Peng et al., 2009; Yan and Ajuwon, 2017).
- *In vivo*
  - Dietary butyrate sodium butyrate improves pig growth performance (Manzanilla et al., 2006; Lu et al., 2008) and tributyrin improved intestinal morphology and barrier function (Hou et al., 2014; Wang et al., 2019).



Objective:

Determine the effects of butyric acid, sodium butyrate, monobutyrin, and tributyrin on transepithelial electrical resistance (TEER) of porcine epithelial cells *in vitro*.

TEER apparatus



https://ebrary.net/24380/health/measurement_transepithelial_electrical_resistance_teer

a) Two electrodes—one on apical and one on basal side of monolayer—measure electrical resistance across monolayer of cells. b) Voltohmeter: Millicell ERS-2 model

Materials and methods

- Culture conditions
  - IPEC-J2 seeded in 12-well transwell plates (Corning) at 5×10^5 cells/mL in DMEM + 5% fetal bovine serum, and 1% penicillin-streptomycin 24 h 37°C 5% CO_2
  - Cells cultured for 4-5 days for monolayer formation (TEER ~1000 Ω) then treated in duplicate
  - TEER measured at 24, 48, and 72 h post-treatment
- Completely Randomized Block Design
  - Dose as fixed effect, plate as random effect
  - Doses: Butyric acid, tributyrin—0, 0.5, 1, 2, 4 mM; Monobutyrin, sodium butyrate—0, 1, 2, 4, 8 mM (determined by MTT assay).

Calculations

Resistance of monolayer

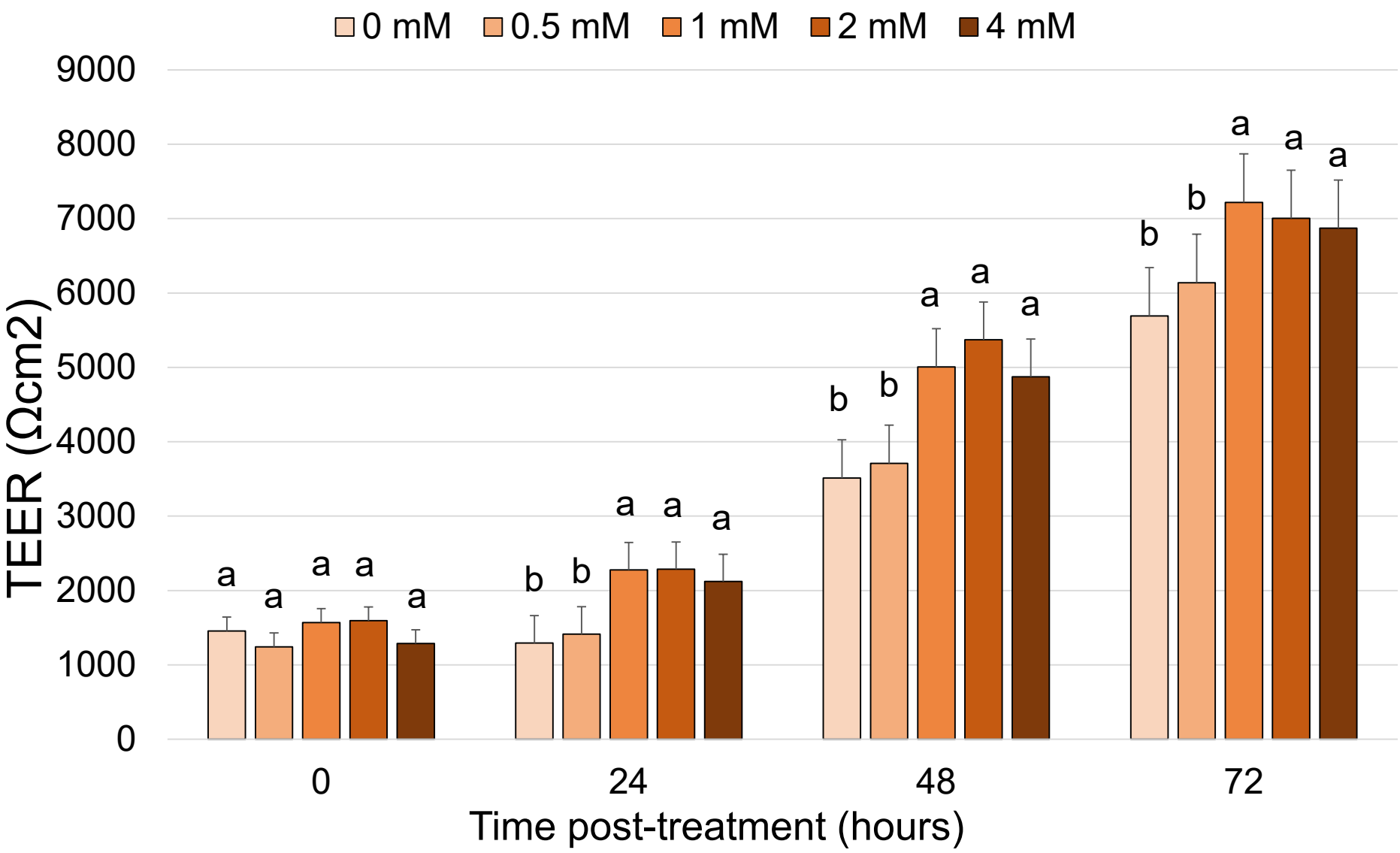
$R_{monolayer}\ [\Omega] = R_{sample} - R_{blank}$

Resistance is inversely proportional to the area of the membrane

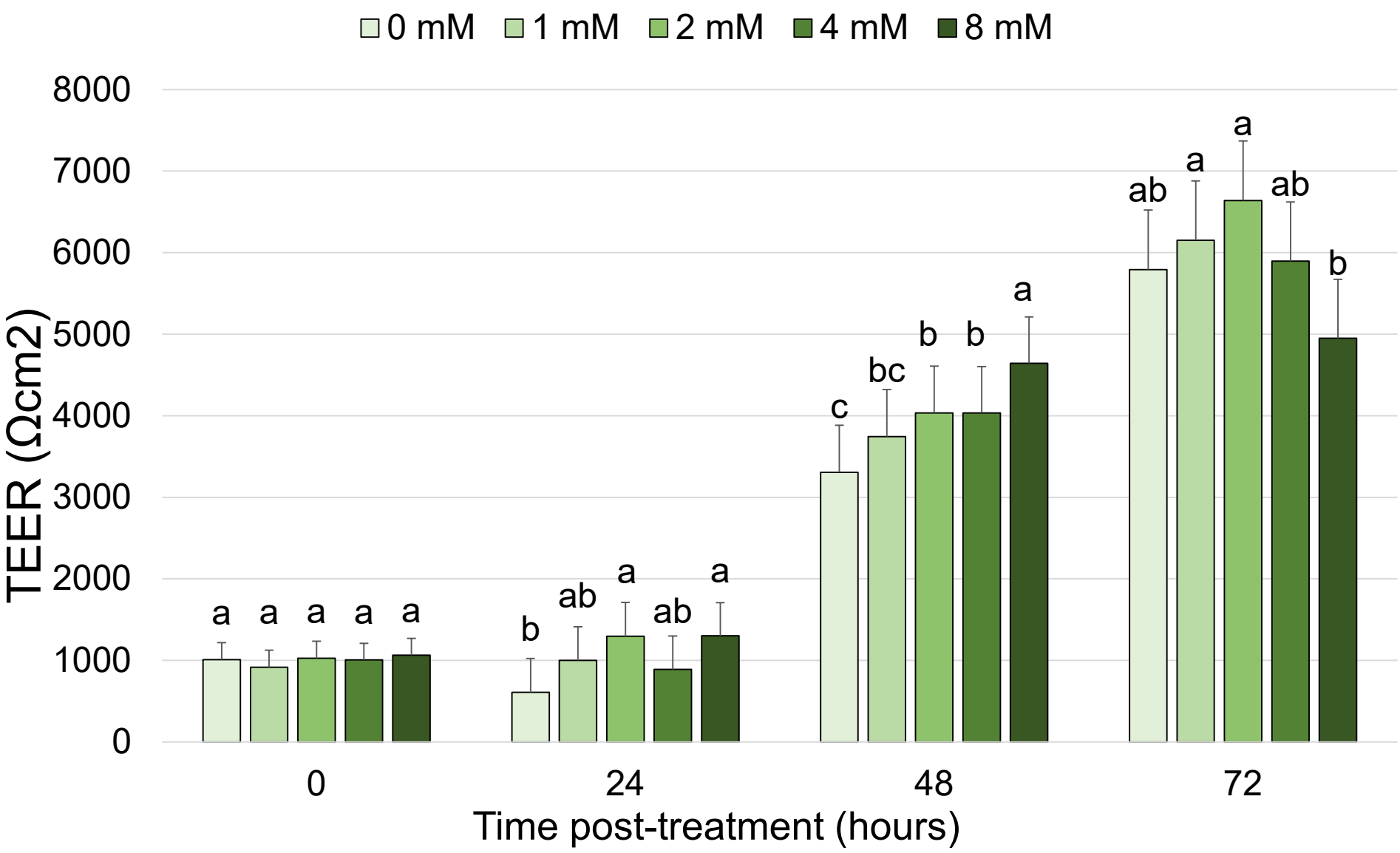
$R_{reported}\ [\Omega cm^2] = R_{monolayer}\ [\Omega] * \text{monolayer area } [cm^2]$

(Srinivasan et al., 2015)

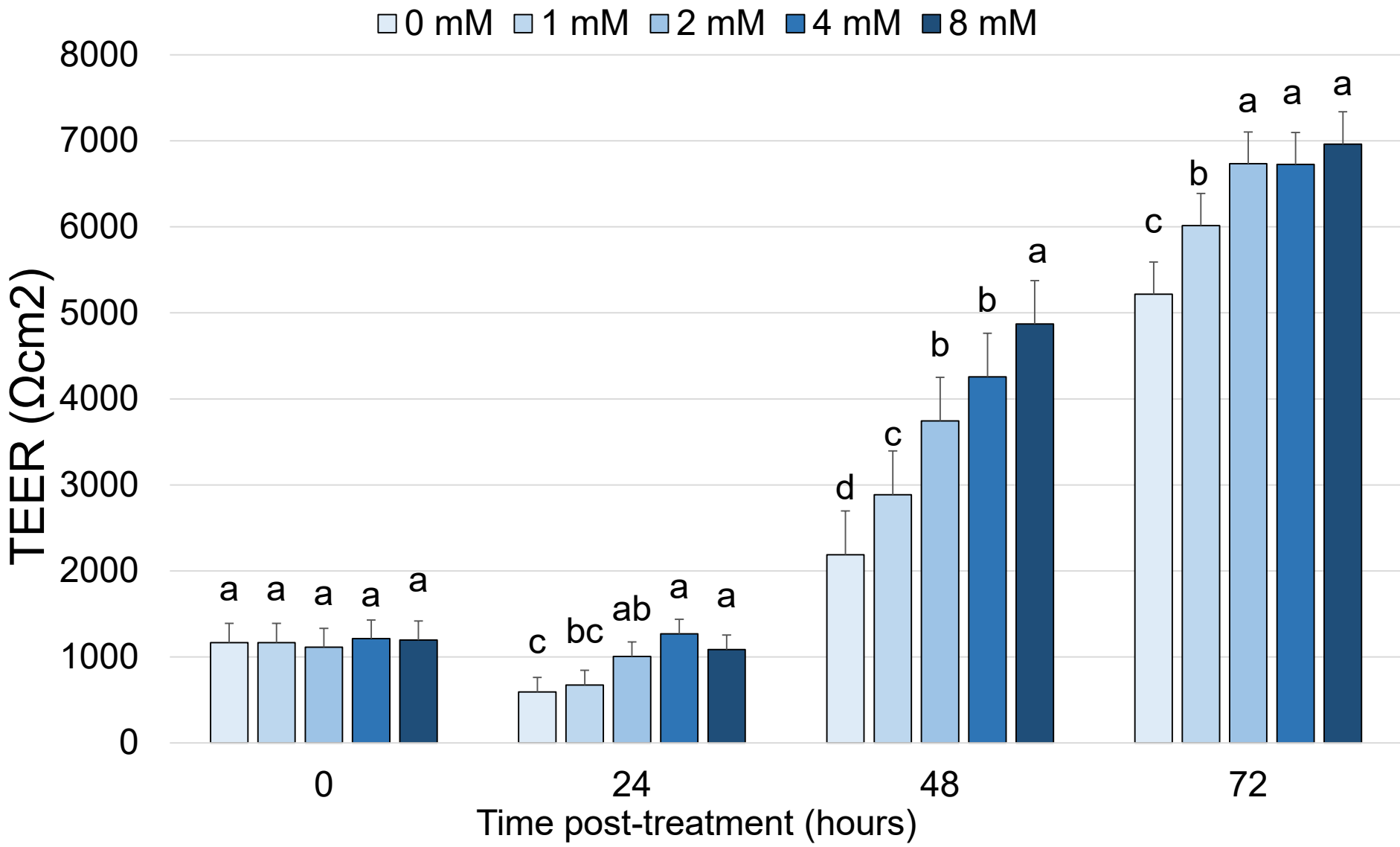
TEER of IPEC-J2 treated with butyric acid



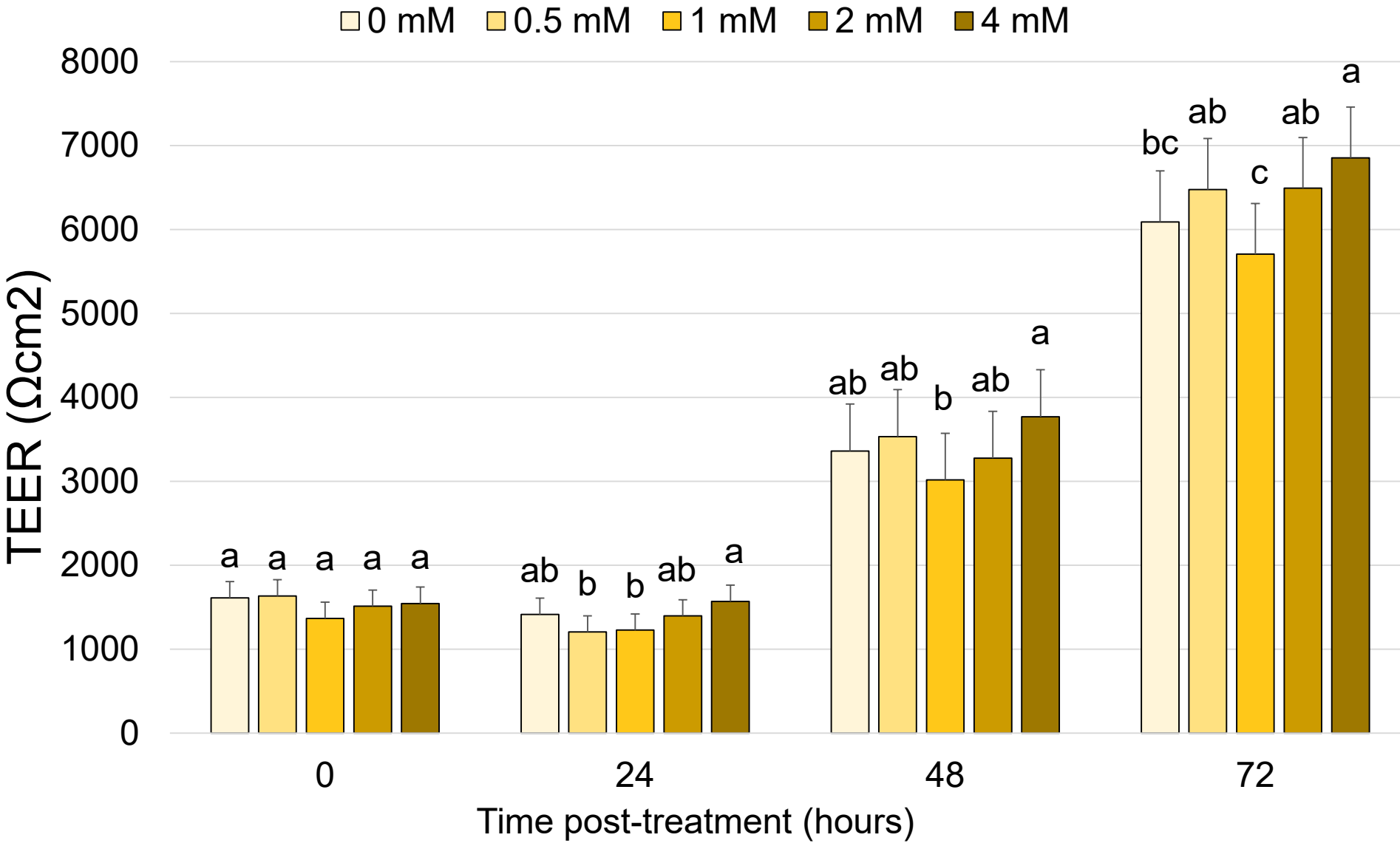
TEER of IPEC-J2 treated with sodium butyrate



TEER of IPEC-J2 treated with monobutyrin



TEER of IPEC-J2 treated with tributyrin



Conclusions

- Butyric acid and monobutyrin increased TEER at all timepoints post-treatment.
- Sodium butyrate increased TEER at 24 and 48 h post-treatment, but highest doses decreased TEER at 72 h post-treatment; 8 mM may be too high.
- Tributyrin tended to increase TEER at highest dose after 72 h with treatment.
  - not cleaved to monobutyrin/butyric acid without presence of lipase, may not affect cells *in vitro*
- ***Butyric acid, monobutyrin, and sodium butyrate increase TEER in porcine epithelial cell monolayers* in vitro**

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