

A Three-Layer Intestinal Model for Toxin Translocation Studies

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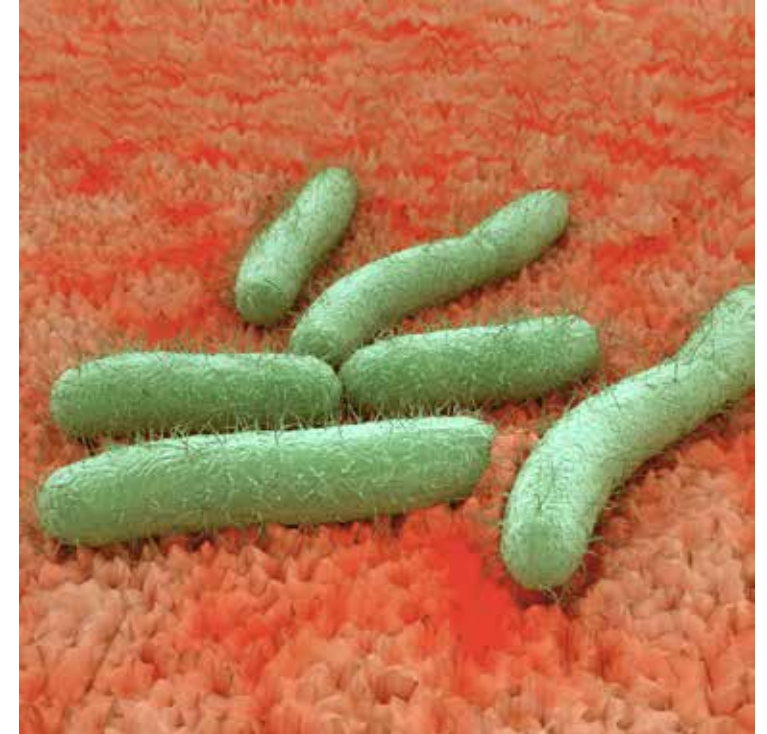
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Shiga Toxin

- Shiga toxin (Stx)-producing E. coli (STEC) are typically ingested from contaminated food or water.
- Serious STEC infections can cause severe abdominal pain, bloody diarrhea, and in 5-15% of cases hemolytic uremic syndrome (HUS).
- There are two immunologically distinct types of Stx (Stx1 and Stx2) - STEC may produce either or both.
- Stxs induce systemic disease by travelling from the intestine through the bloodstream to localized sites such as the kidney
- No specific therapy is currently available to treat STEC-induced HUS. A further understanding of the mechanisms by which Stx crosses the intestinal epithelial barrier may lead to additional treatment approaches.

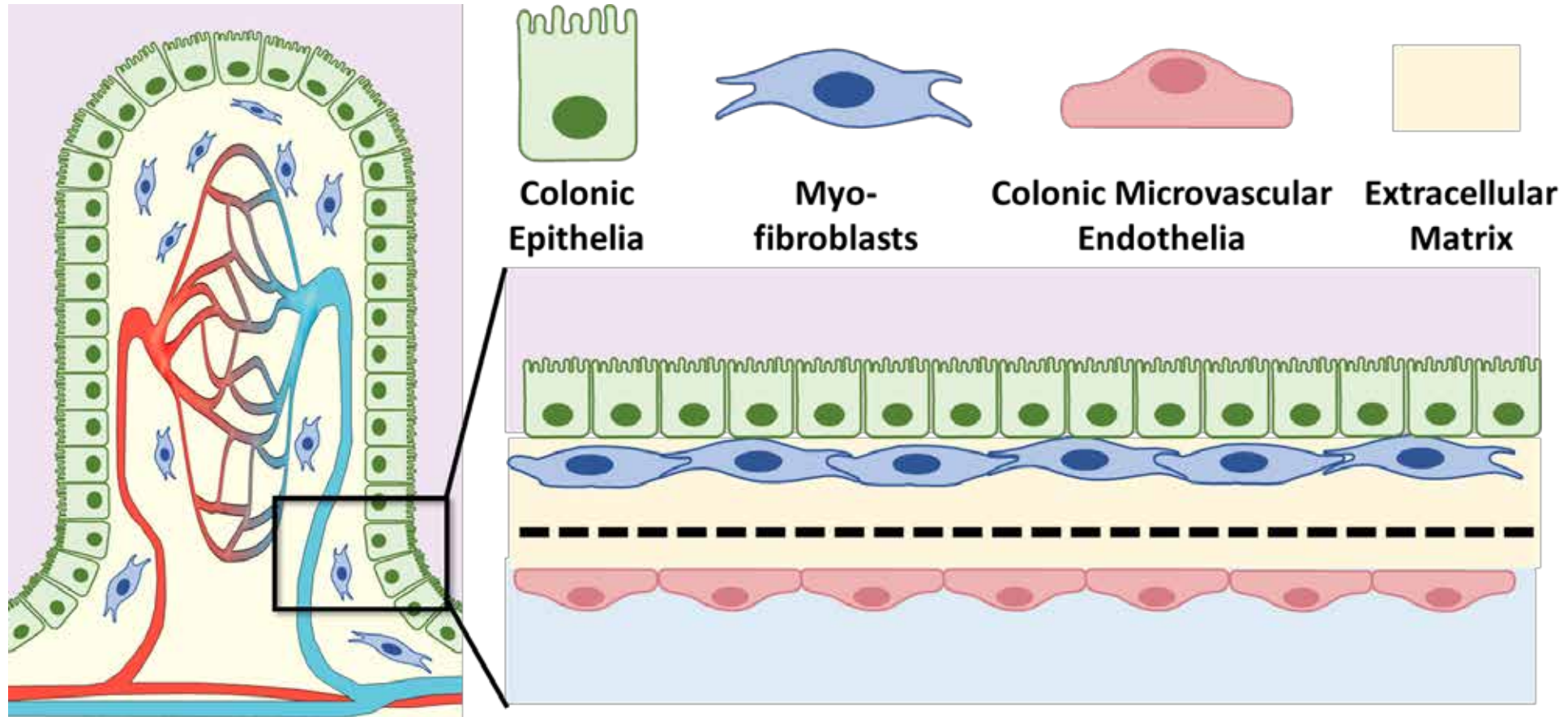


<https://mb-labs.com/testlibrary/stec/>

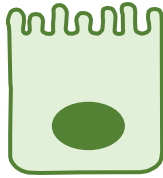
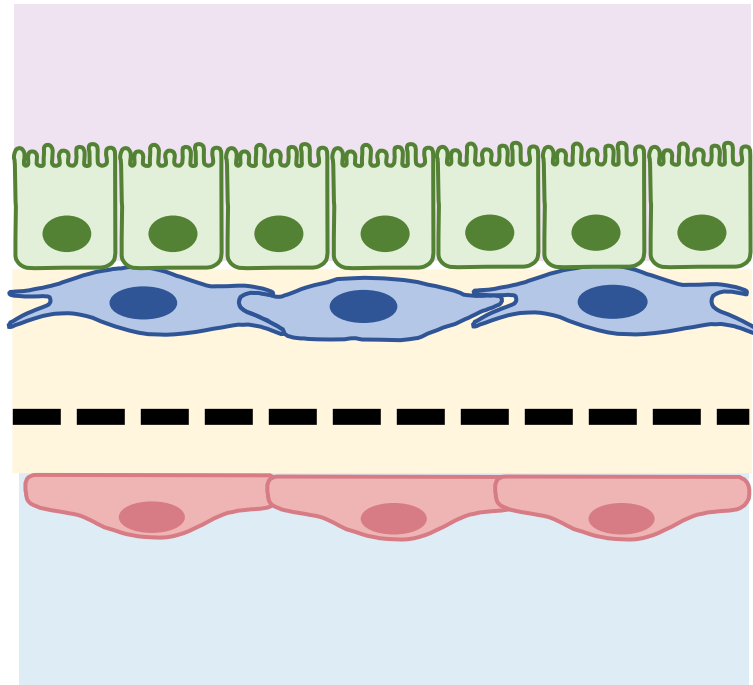
In Vitro Studies of Stx Transport

- Most of the knowledge of Stx transport has been derived using mono-layers of immortalized intestinal epithelial cells (e.g., Caco-2, HCT-8, T84)
- Transcellular transport is the most common mechanism reported for Stx movement through the intestinal epithelium
- Conflicting evidence of uptake by micropinocytosis
- Paracellular transport pathways have also been reported – (1) direct damage to the mucosa and epithelial barrier and (2) trafficking of Stx by immune cells through the epithelial barrier.
- In summary, Stxs may employ different methods to traverse the intestinal epithelium and inconsistencies may be due to variability of the in vitro models
- There is evidence that Stx behavior is modified in tissue models with greater physiological complexity than cultured monolayers.
- Recent work with human intestinal organoids demonstrated that mesenchymal cells in the HIOs play an important physiological role for the epithelial barrier.

Multilayer Tissue Model



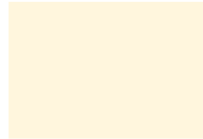
Multilayer Model



Colonic Epithelia: Human primary colonic epithelial cells (Cell Biologics)



Myofibroblasts: Differentiated from human adipose-derived mesenchymal stem cells

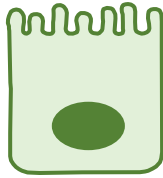
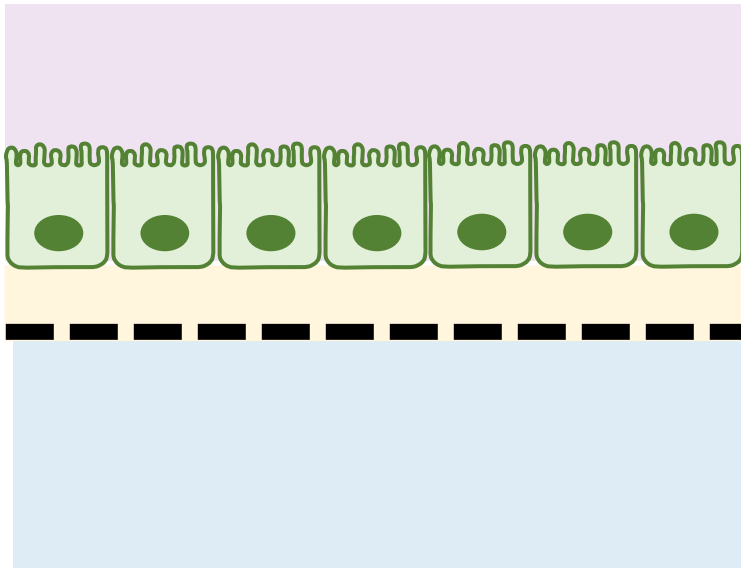


PET Membrane (1 um pore): collagen/gelatin on apical side, gelatin coating on basal side

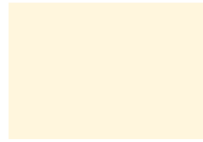


Colonic Microvascular Endothelia: Human primary colonic microvascular endothelial cells (Cell Biologics)

Single-layer Model for Comparison



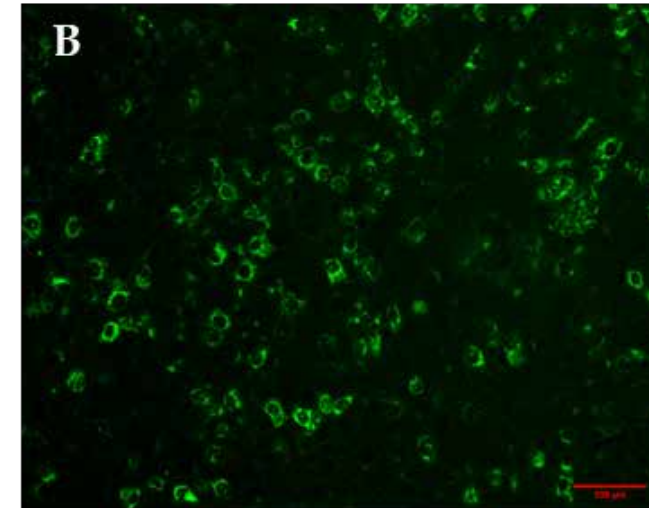
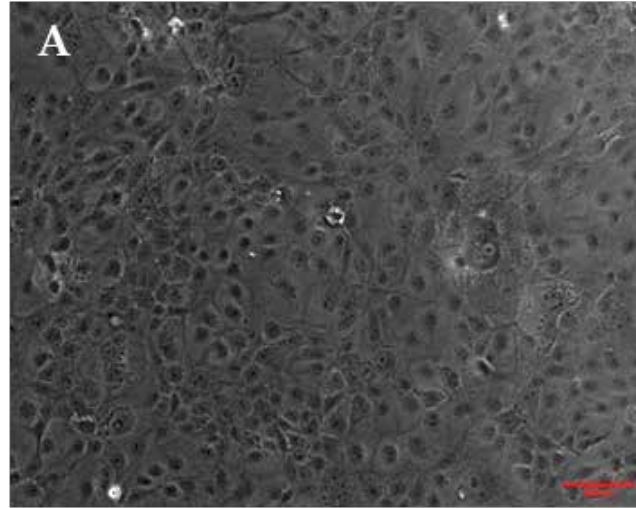
Colonic Epithelia: Human primary colonic epithelial cells (Cell Biologics)



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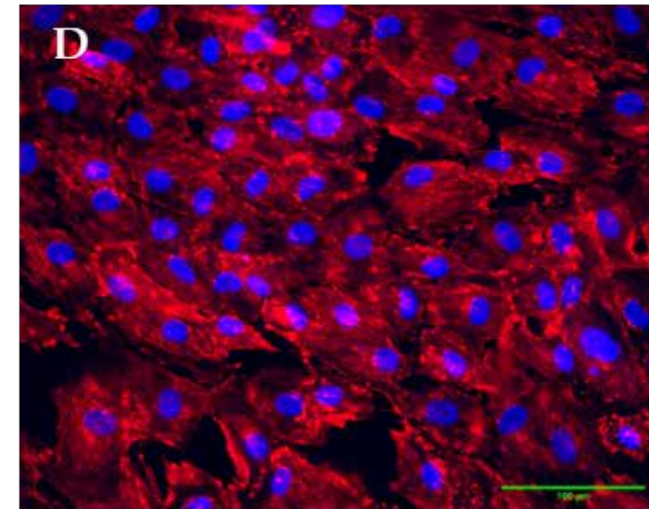
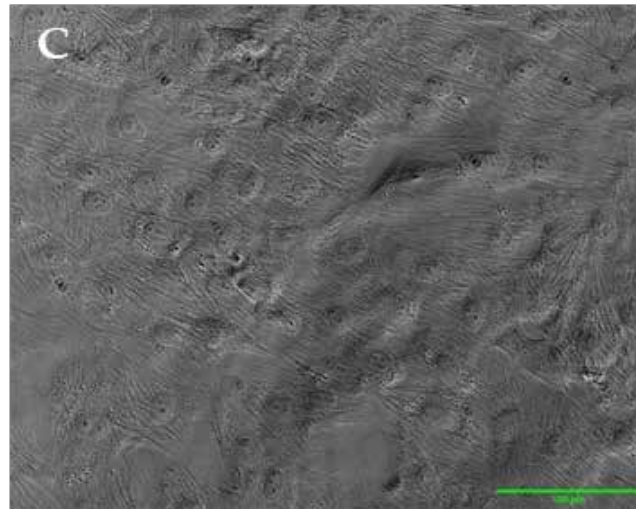
Monolayer Cell Validation

Primary human colonic epithelial cells express Muc5b, a mucin precursor common to intestinal epithelia.



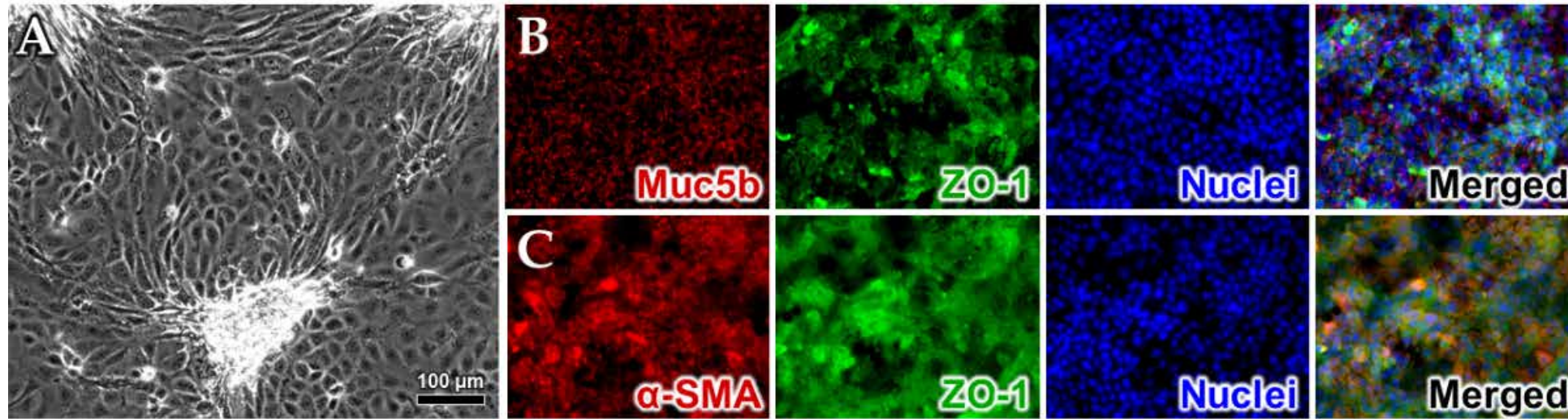
anti-Muc5B

hMSC-derived myofibroblasts demonstrate significant alpha-smooth muscle actin, a marker of mature fibroblasts



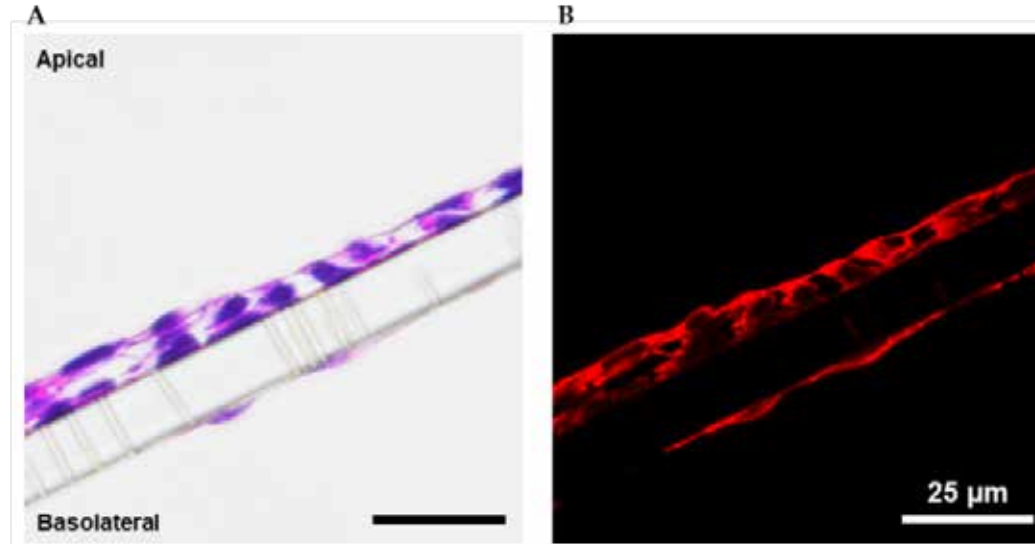
anti-alpha
SMA
DAPI

Myofibroblast and epithelial co-culture validation



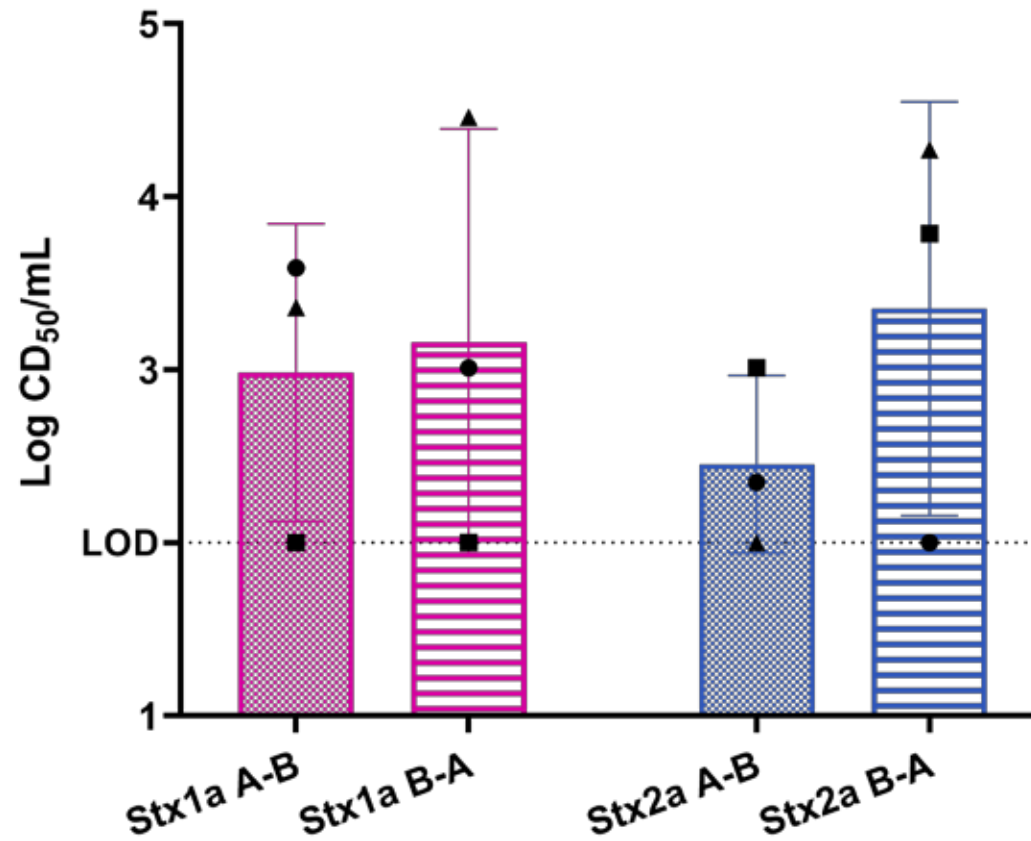
- Differentiated myofibroblasts seeded onto a transwell membrane and cultured to 80-90% confluence
- Primary colonic epithelia seeded directly over top
- 3:1 mixture of epithelial cell media and myofibroblast differentiation media sustained cell viability during co-culture for at least 2 weeks
- Epithelial cells generated a cobblestone-like monolayer while myofibroblasts maintained a stretched morphology

Tri-layer Model

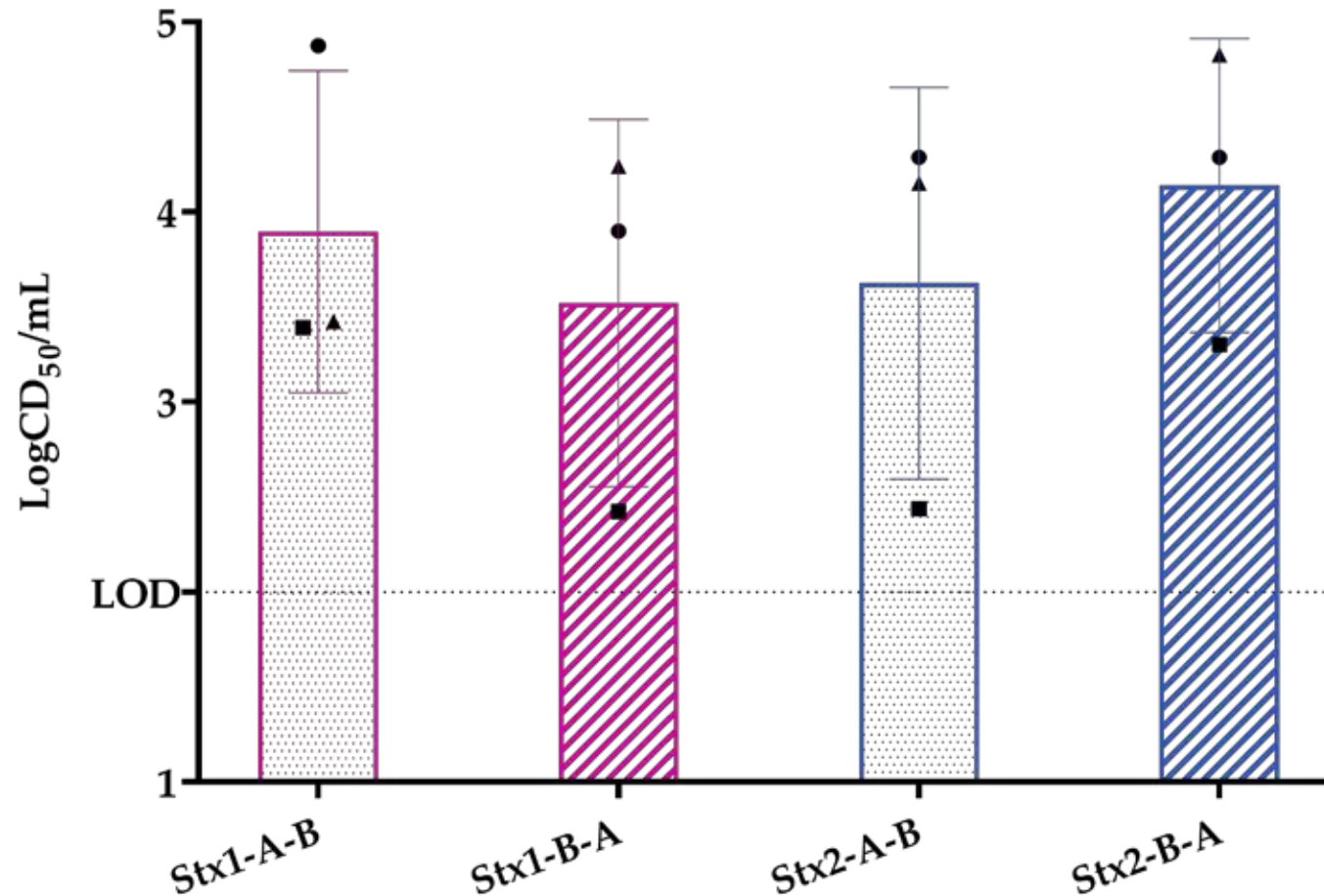


- Endothelial cells seeded on inverted transwells
- After 2 hours, flipped over and myofibroblasts seeded on apical side
- After 5 days, epithelial cells seeded on apical side
- Experiments performed 7-10 days after epithelial cell seeding
- Histology verified dual cell layer on the apical side and a thin layer on the basolateral side of the membrane

Stx1 and Stx2 translocate similarly – single layer



10-fold more Stx1 and Stx2 translocate – triple layer

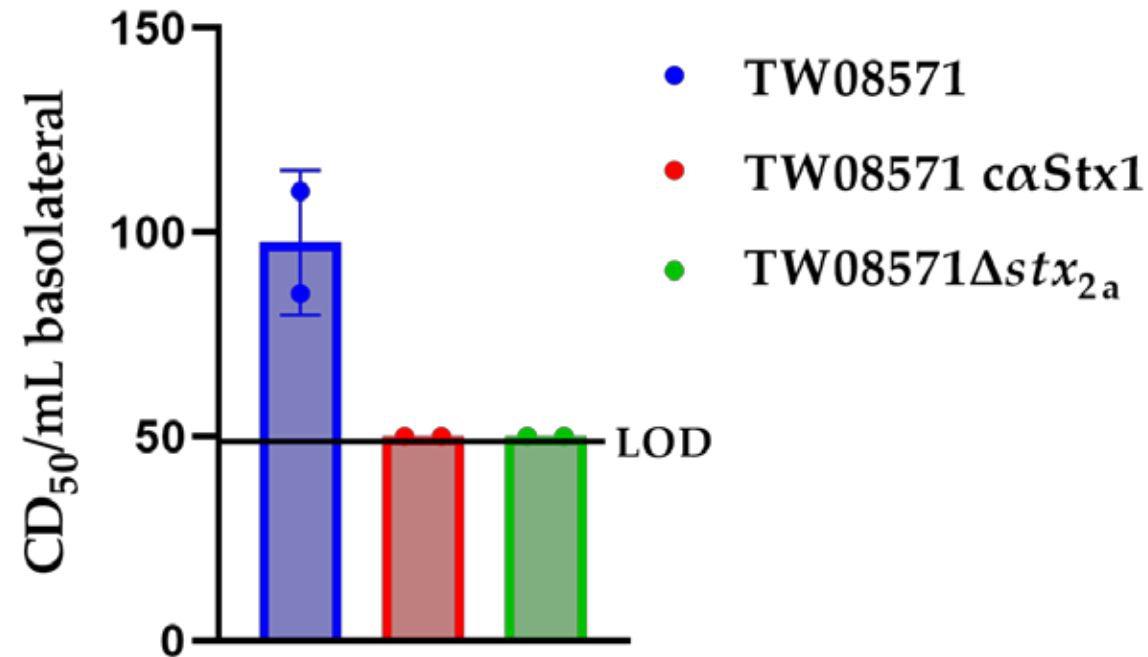


Toxin translocation, triple layer – STEC

1. STEC O157:H7 – Stx2 producer, *eae*+
2. STEC O26:H11 – Stx1, Stx2, *eae*+

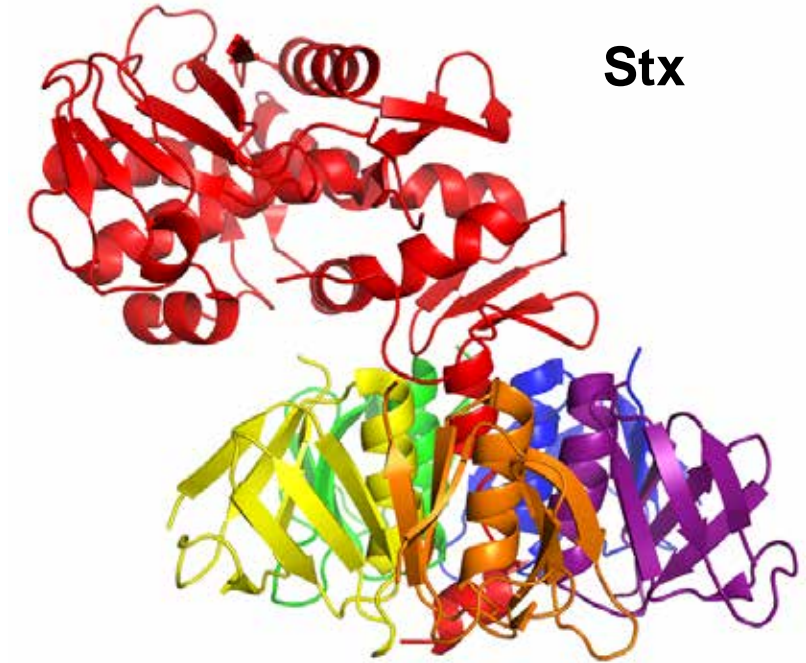
- No basolateral toxin at 6 h post-infection
- O157:H7 strain – destroyed model by 18 h pi
 - *eae* mutant – destroyed model

Low levels of toxin translocation – STEC



Summary

- Single and triple layer models were developed.
- Both Stx1 and Stx2 translocated similarly across the models
- The three-layer model showed 10-fold toxin translocation than the single-layer model.
- Future studies will focus on furthering our understanding of the three-layer model and how toxin is translocating in that model.



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