Nuove metodologie per lo studio dell’interazione ospite - patogeno

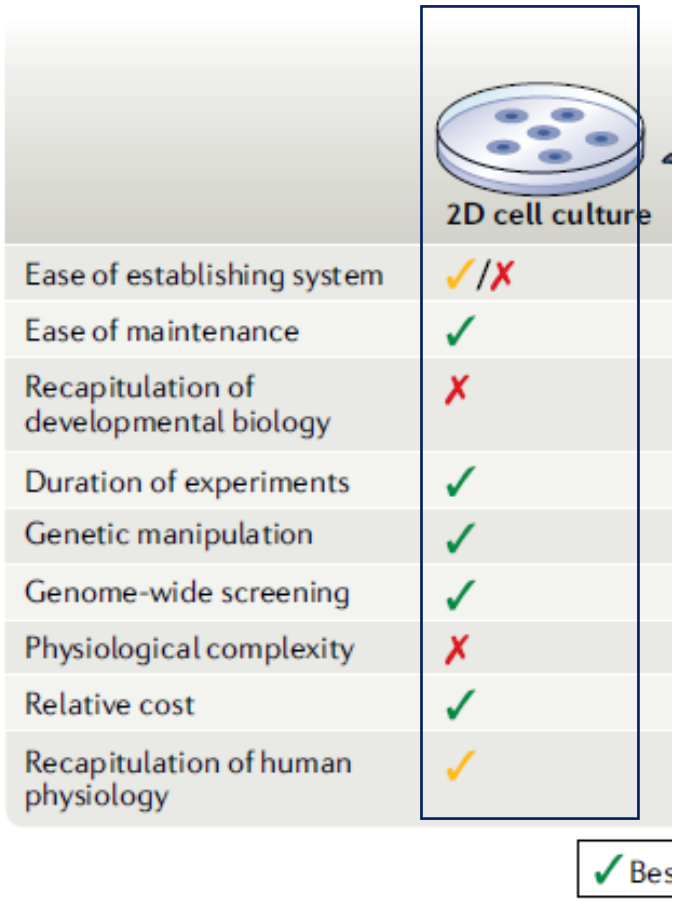
Model systems for human biology

	 2D cell culture	 *C.elegans*	 *D. melanogaster*	 *D. rerio*	 *M. musculus*	 PDX
Ease of establishing system	✓/✗	✓	✓	✓	✓	✓
Ease of maintenance	✓	✓	✓	✓	✓	✓
Recapitulation of developmental biology	✗	✓	✓	✓	✓	✗
Duration of experiments	✓	✓	✓	✓	✓	✓
Genetic manipulation	✓	✓	✓	✓	✓	✗
Genome-wide screening	✓	✓	✓	✓	✗	✗
Physiological complexity	✗	✓	✓	✓	✓	✓
Relative cost	✓	✓	✓	✓	✓	✓
Recapitulation of human physiology	✓	✓	✓	✓	✓	✓

✓ Best ✓ Good ✓ Partly suitable ✗ Not suitable

Kim et al., 2020

Use of cell lines to study host-pathogen interactions



	2D cell culture
Ease of establishing system	✓/✗
Ease of maintenance	✓
Recapitulation of developmental biology	✗
Duration of experiments	✓
Genetic manipulation	✓
Genome-wide screening	✓
Physiological complexity	✗
Relative cost	✓
Recapitulation of human physiology	✓

✓ Bes

ADVANTAGES

Immortalized cell lines remain the work horse of in vitro intestinal models due to their relative ease of culture, low-cost, and potential for scale up.

DISADVANTAGES

Cell lines are not able to truly mimic all of the subsets of epithelial cells found in vivo.

However, the application may not entirely necessitate a physiologically complete model. When trying to elucidate fundamental mechanisms it is often useful to begin with a well characterized and understood system in a reductionist manner and later build up complexity.

Use of cell lines to study host-pathogen interactions

Table 4. A summary of cell lines commonly used for in vitro models of the intestinal epithelium.

Cell line	Characteristics	Origin	Application	Ref
Caco-2	Cylindrical polarised monolayer, apical brush border with enzyme secreting microvilli, tight junctions between adjacent cells, forms domes	Human colon adenocarcinoma	Barrier models, permeability assays, drug absorption, metabolism, 3D cell culture, toxicology	[144–146]
HT-29	Cylindrical polarised monolayer, apical brush border with enzyme secreting microvilli, tight junctions between adjacent cells HT29-MTX are mucin producing and goblet cell-like	Human colon adenocarcinoma	Barrier models, drug absorption, metabolism, cancer research, 3D cell culture, toxicology, mucus extraction	[147–149]
T84	Like Caco-2 but with more colonocyte-like features including shorter microvilli. Generally, exhibit higher TEER than Caco-2.	Human colon carcinoma (lung metastasis)	Cancer research, neurotransmitter and hormone studies, barrier models	[150–152]
SW480	Heterogenous cell population producing polygonal cells of the typical epithelial type (E-type) and round refractile cells (R-type). R-type forms multilayers. Large microvilli and glycogen stores. Deep processes observed through inserts	Human colon adenocarcinoma	Cancer research, 3D cell culture, drug delivery, migration studies	[153–155]
IEC-6	Normal cell type. Synthesise fibronectin and collagen. Tight colonies with polygonal shape. Crypt cell characteristics and tight junctions.	Rat small intestine epithelia	3D cell culture, transport studies, ECM, parasites, healthy cell metabolism	[156–158]
HCT-8	Heterogenous organisation and small proportion of cells expressing SI, villin and ZO1. Methotrexate-selected HCT8-MTX cells show universal expression of ZO1 and MUC1.	Human ileocecal adenocarcinoma	3D cell culture, cancer research, toxicology, virus entry, parasite,	[159–162]
FHC	Normal cell type. Exhibits tumorigenic phenotype.	Foetal colonic epithelial	3D cell culture, drug delivery, cancer research, foetal research, uptake	[163, 164]
Raji	Grow as single cells without attachment. Clumps may form. Carries latent Epstein-Barr Virus.	Human B lymphoblastoid	Toxicity, 3D cell culture, immunology	[139, 140, 165, 166]
THP-1	Grown in suspension. Can be stimulated by phorbol-12-myristate-13-acetate (PMA) into macrophage-like cells	Human monocytic leukaemia	3D cell culture, immunology, toxicology	[167–169]

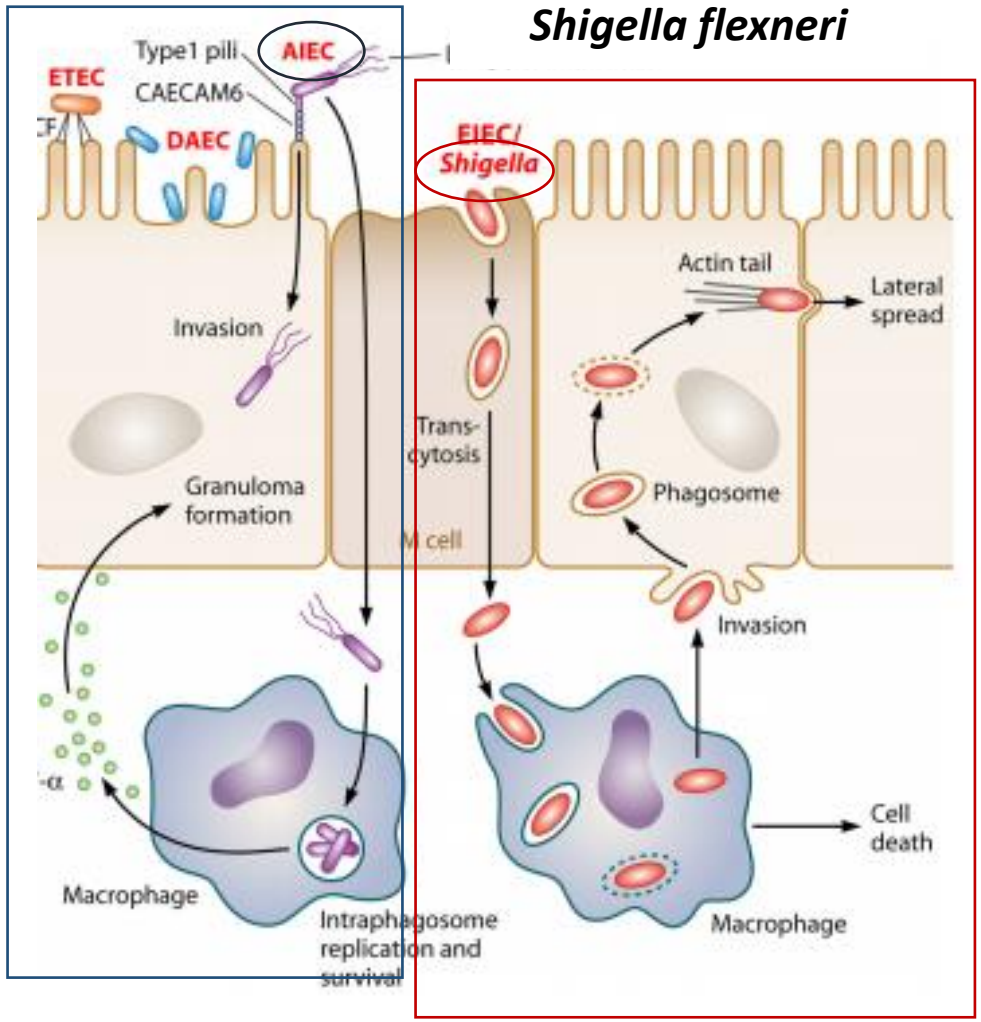
McCoy et al., 2023

Enteropathogen *E. coli* we will use as model systems

- Associated with Crohn's disease (CD)
- Persist and multiply intracellularly in **epithelial cells** (in late endosomes) and in **macrophages** (inside maturing phagolysosomes)
- Reference strain **LF82**

Adherent Invasive *E. coli*

Shigella flexneri



- Now considered as enteroinvasive *E. coli*
- Invades **macrophages** and induces rapid cell death;
- Invade from the basolater side **enterocytes**, where intracellular replication and dissemination occurs;
- Invasive program is **regulated** in response to environmental signals (pH, temperature, osmolarity, iron)
- Reference strain **M90T**

Croxen *et al*., 2013

Typical experimental workflow

Costruction of *S. flexneri* M90T or AIEC LF82 mutant strains by:

- one-step inactivation of chromosomal genes (Datsenko and Wanner, 2000)
- P1 transduction (from *E. coli* mutant strains)

+

Prepation of cell line as model for bacterial infection:

- Cell culturing
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S. flexneri / AIEC infection of

- macrophages (up to 3h): THP-1 monocytes differentiated into macrophages
- epithelial cells (up to 4h): human epithelial colorectal adenocarcinoma Caco-2 cell line

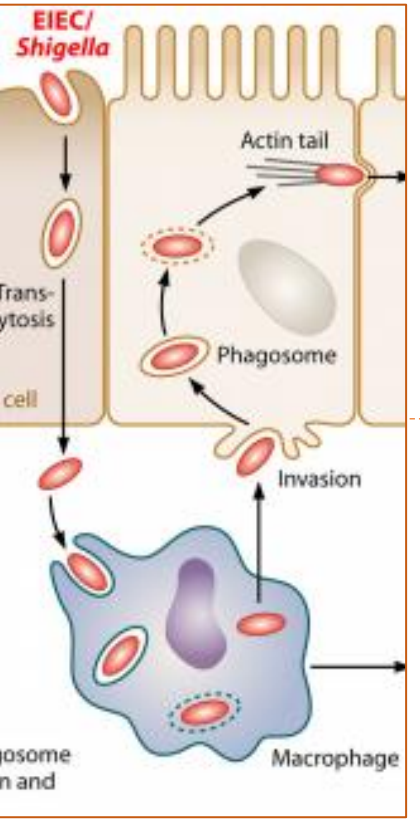
Recovery of intracellular bacteria after cell lysis

- Bacterial RNA extraction and evaluation of target gene expression by Real Time PCR
- Evaluation of intracellular survival by:
  - Live and Death Assay (DAPI/ PI double staining)
  - Viable Bacterial Count (CFU/ml)

Recovery of infected cells supernatant

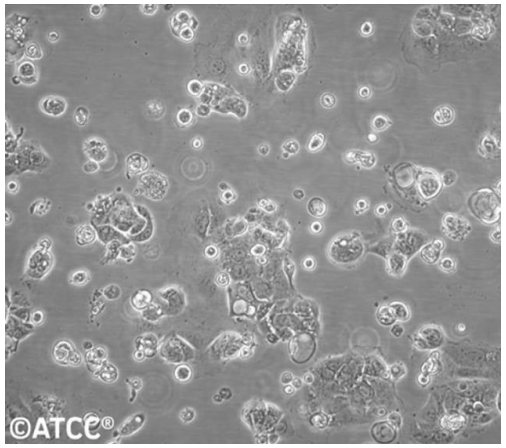
- Cytotoxicity of host cells after bacterial infection by LDH cytotoxicity assay
- Host immune response after bacterial infection by ELISA assays

How to prepare cell line as model to be infected by enteropathogens



epithelial cells

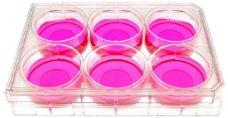
Caco-2 cells (low density)



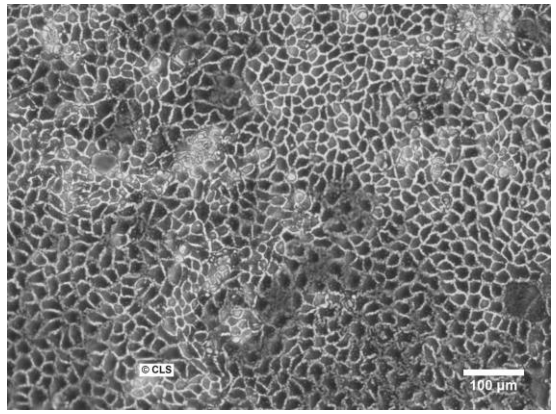
- Assessing cell density
- Cell counting (burker chamber)
- Calculate the number of cells to be plated

Complete medium DF10 → 24h

Serum starvation medium DF0,5 → 24h

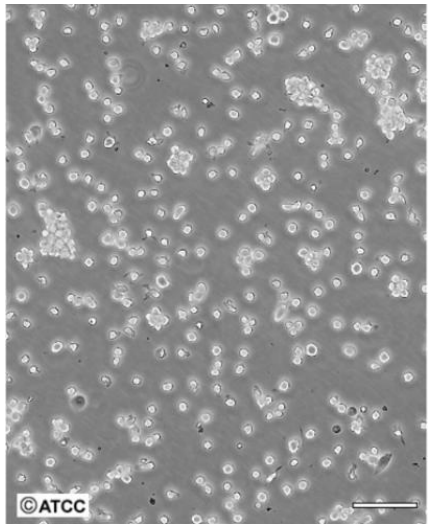


Caco-2 cells (high density)



macrophages

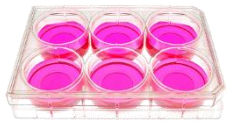
THP-1 monocytes



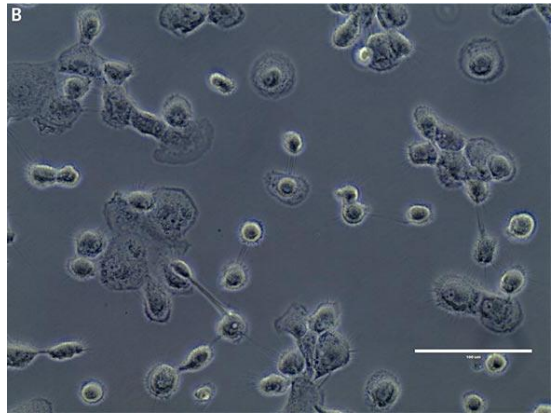
- Assessing cell health
- Cell counting (burker chamber)
- Calculate the number of cells to be plated

Monocytic differentiation into macrophages
+ phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA)

→ 72h



THP-1-derived macrophages



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sample fixation and observation at the microscope

Recovery of infected cells supernatant

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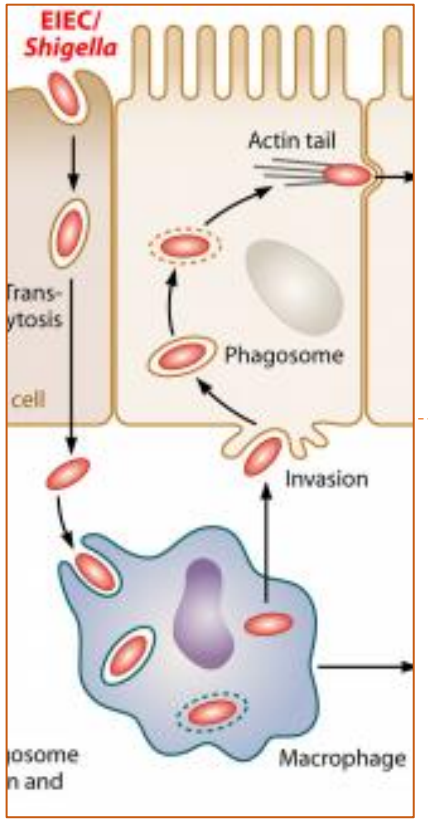
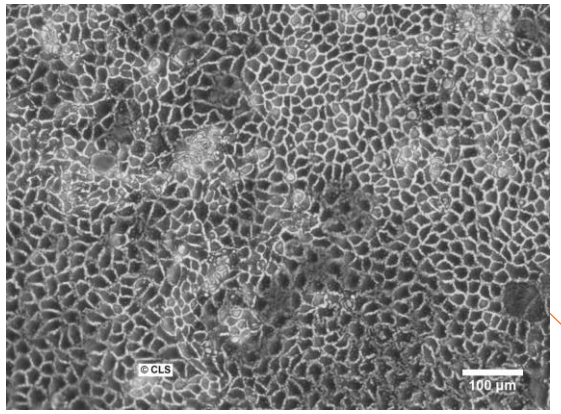
Workflow for macrophage and epithelial cell infection experiments



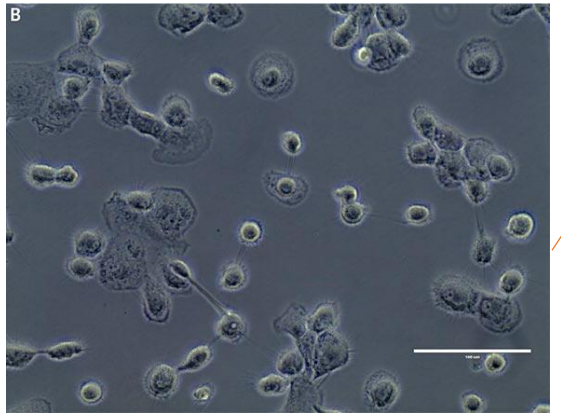
Caco-2 epithelial cells

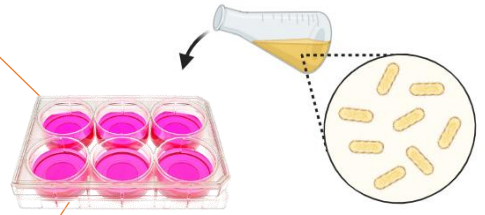
epithelial cells



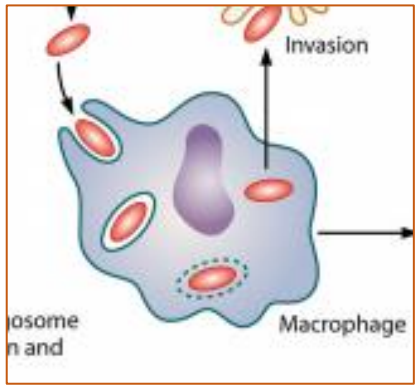
THP-1-derived macrophages

macrophages





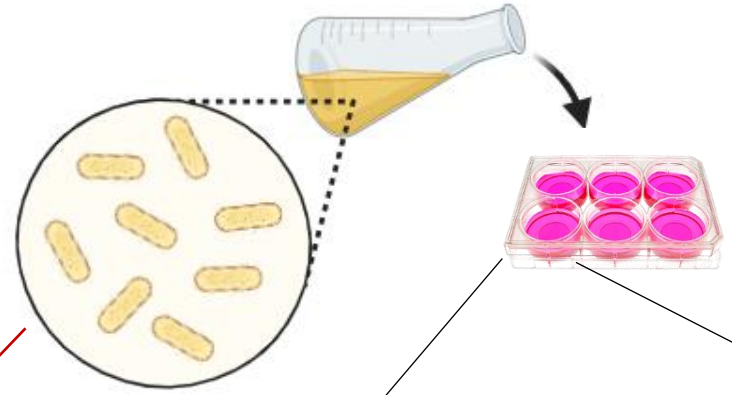
Infection of macrophages (THP1) and epithelial cells (Caco-2) up to 3h and 4h, respectively.



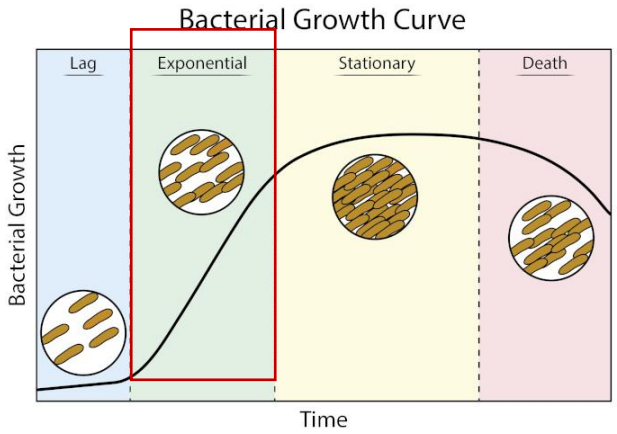
Macrophage infection experiment

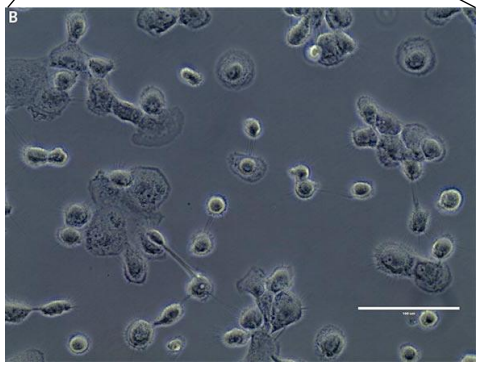
3. Addition of bacteria to seeded cells considering:

- Number of cell/well
- MOI



1. Bacterial growth until exponential phase





2. Preparation of cells before infection

4. Incubation at 37°C in a humidified 5% CO2 atmosphere 30 minutes

5. Wash away extracellular bacteria and add new culture media supplemented with gentamicin (kills extracellular bacteria).

6. Infection times start

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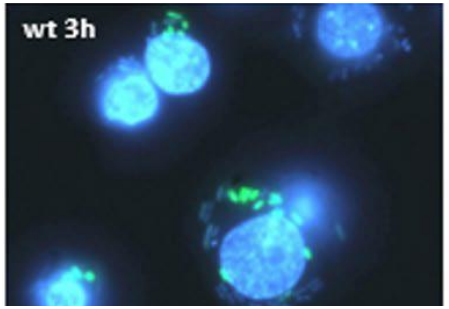

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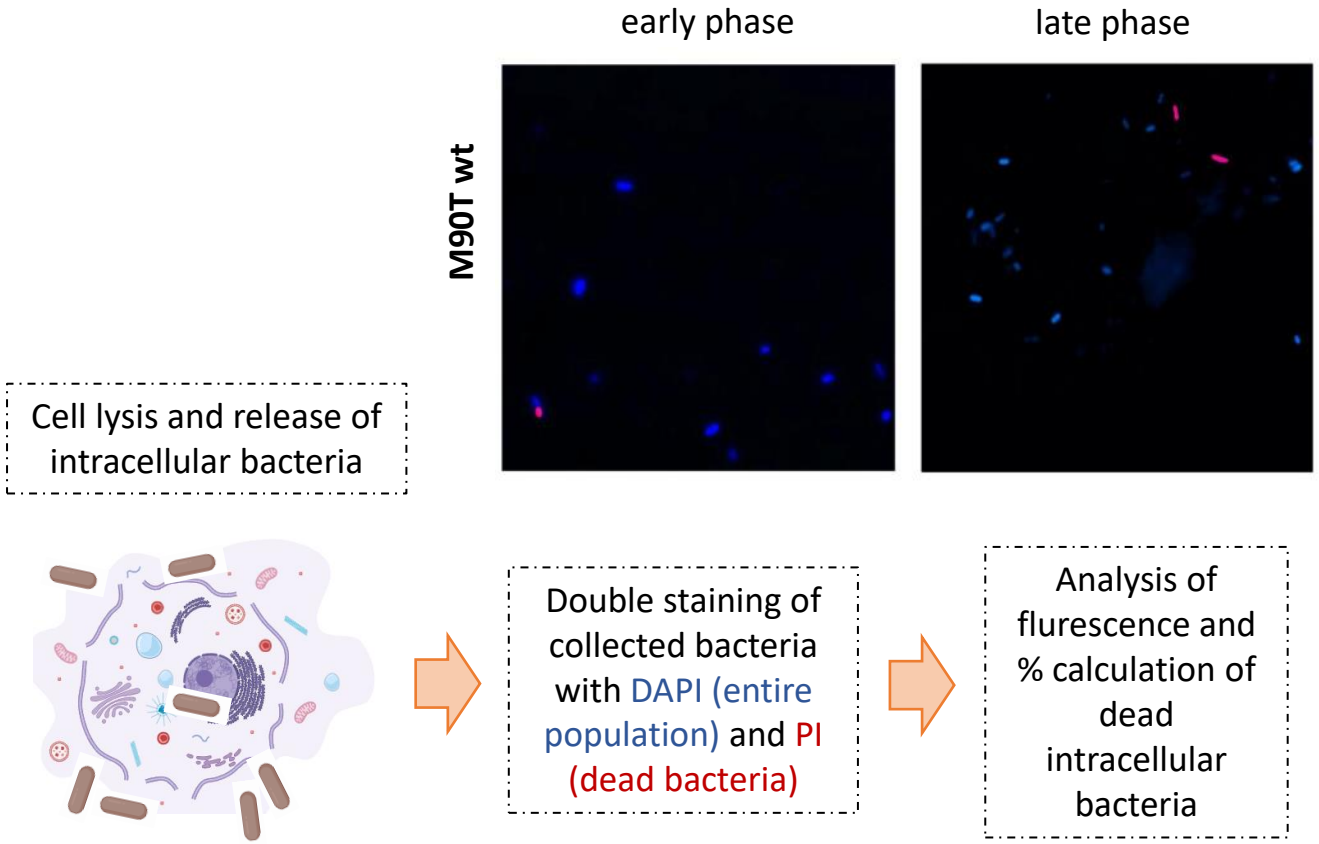
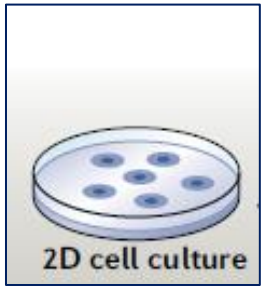
Recovery of intracellular bacteria after cell lysis

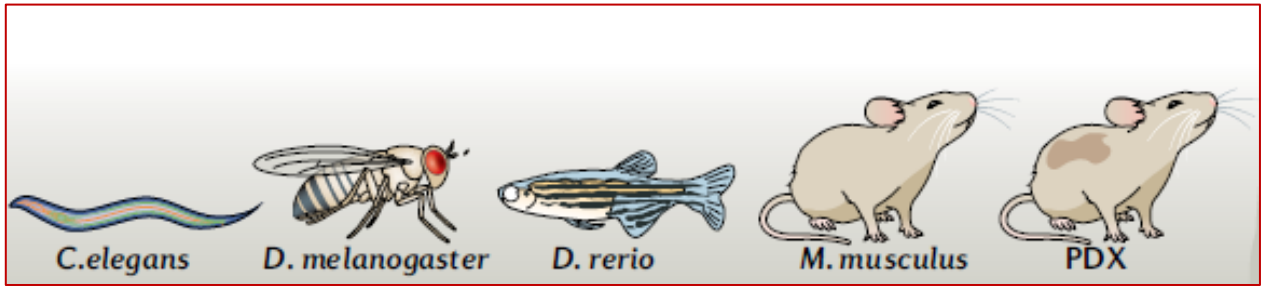
Evaluation of intracellular survival



Limitations of models used in studies on the pathogenesis of intestinal bacteria

Fail to depict the genuine human response accurately





<u>Cell lines</u>

Immortalized or transformed, such as Caco-2, are not reflective of normal physiological conditions due to their altered genetic phenotype

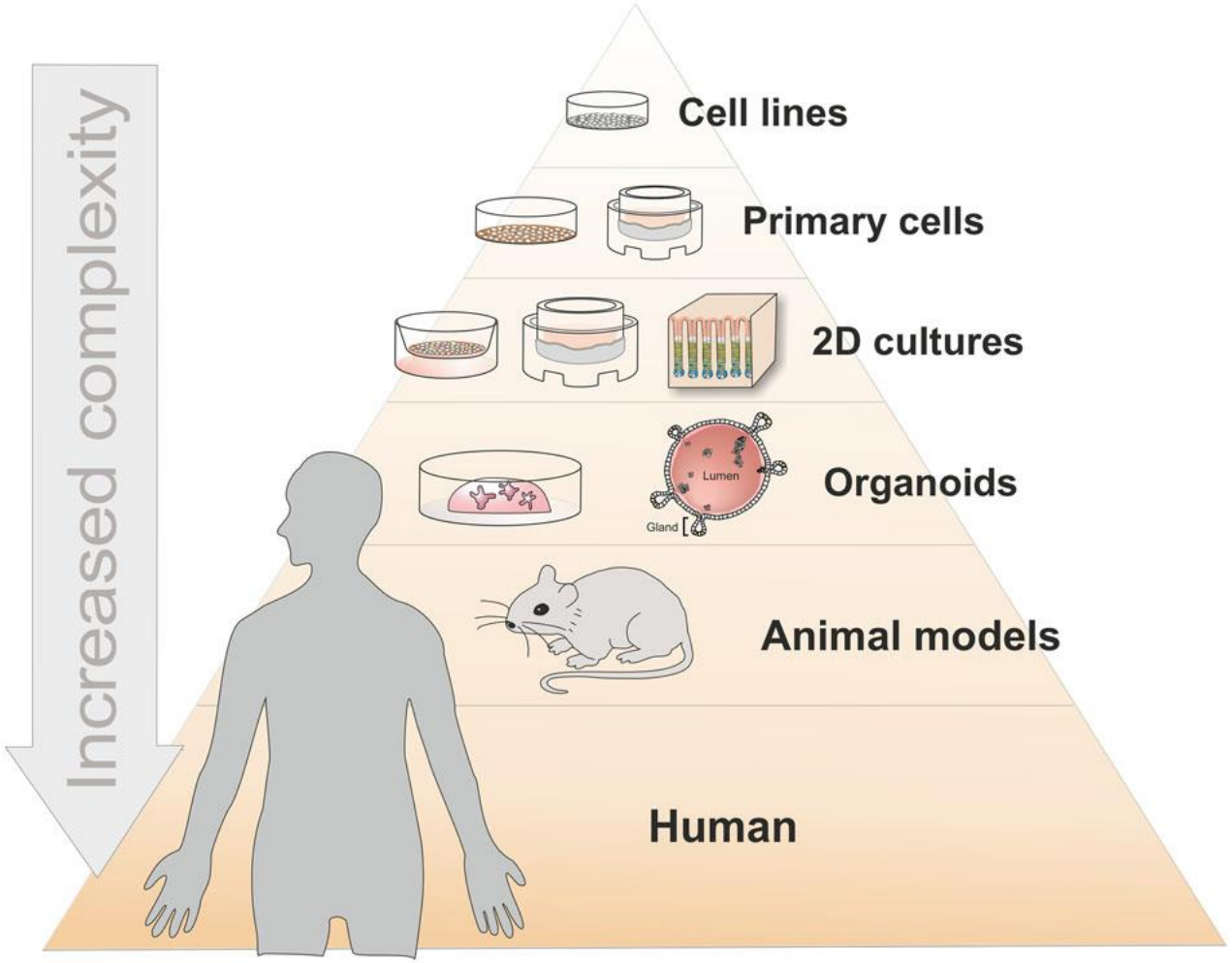
composition of single cell type

<u>Animal models</u>

such as mice do not share identical biological and histologic traits with humans, and may differ greatly from humans concerning the immune system

More precise models are imperatively required to develop more accurate studies on host-pathogen interactions and drug discovery.

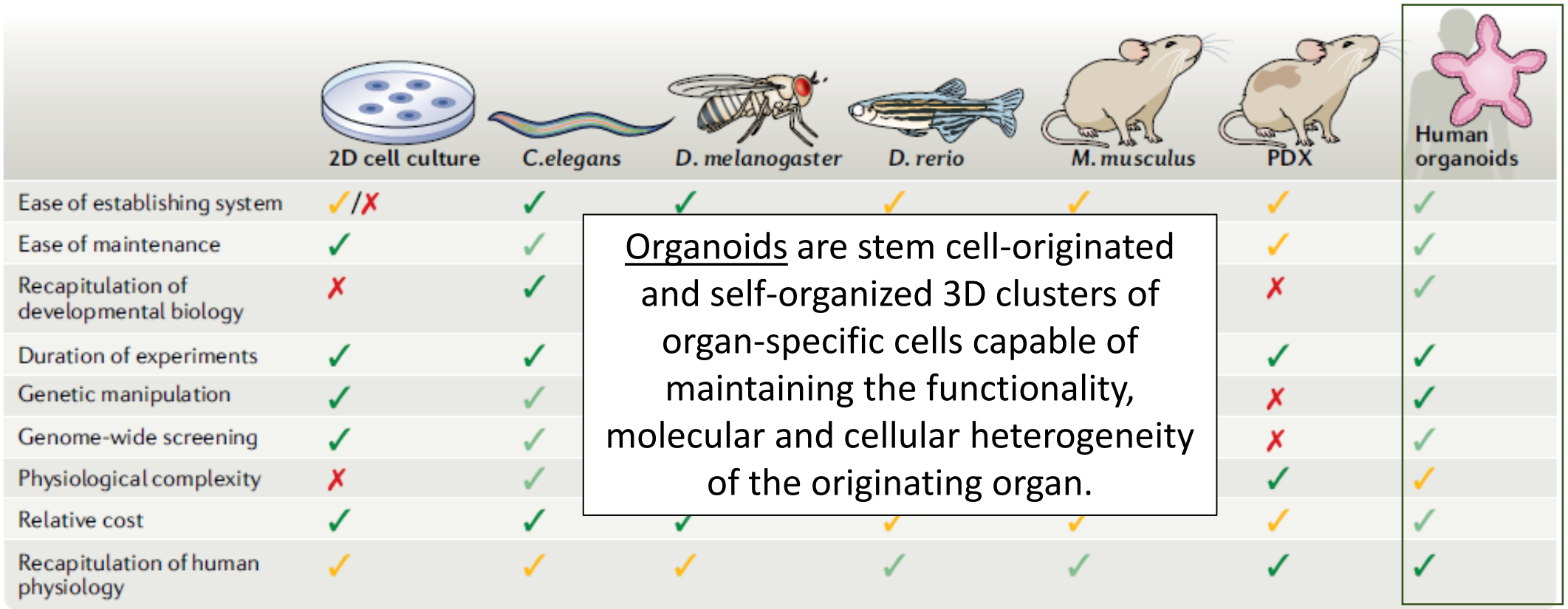
Advancements in understanding bacterial enteritis pathogenesis



Aguilar et al., 2021

Model systems for human biology

The advent of human organoids it is now possible to re-create the architecture and physiology of human organs in remarkable detail



	2D cell culture	*C.elegans*	*D. melanogaster*	*D. rerio*	*M. musculus*	PDX	Human organoids
Ease of establishing system	✓/✗	✓	✓	✓	✓	✓	✓
Ease of maintenance	✓	✓				✓	✓
Recapitulation of developmental biology	✗	✓				✗	✓
Duration of experiments	✓	✓				✓	✓
Genetic manipulation	✓	✓				✗	✓
Genome-wide screening	✓	✓				✗	✓
Physiological complexity	✗	✓				✓	✓
Relative cost	✓	✓	✓	✓	✓	✓	✓
Recapitulation of human physiology	✓	✓	✓	✓	✓	✓	✓

✓ Best ✓ Good ✓ Partly suitable ✗ Not suitable

Kim et al., 2020

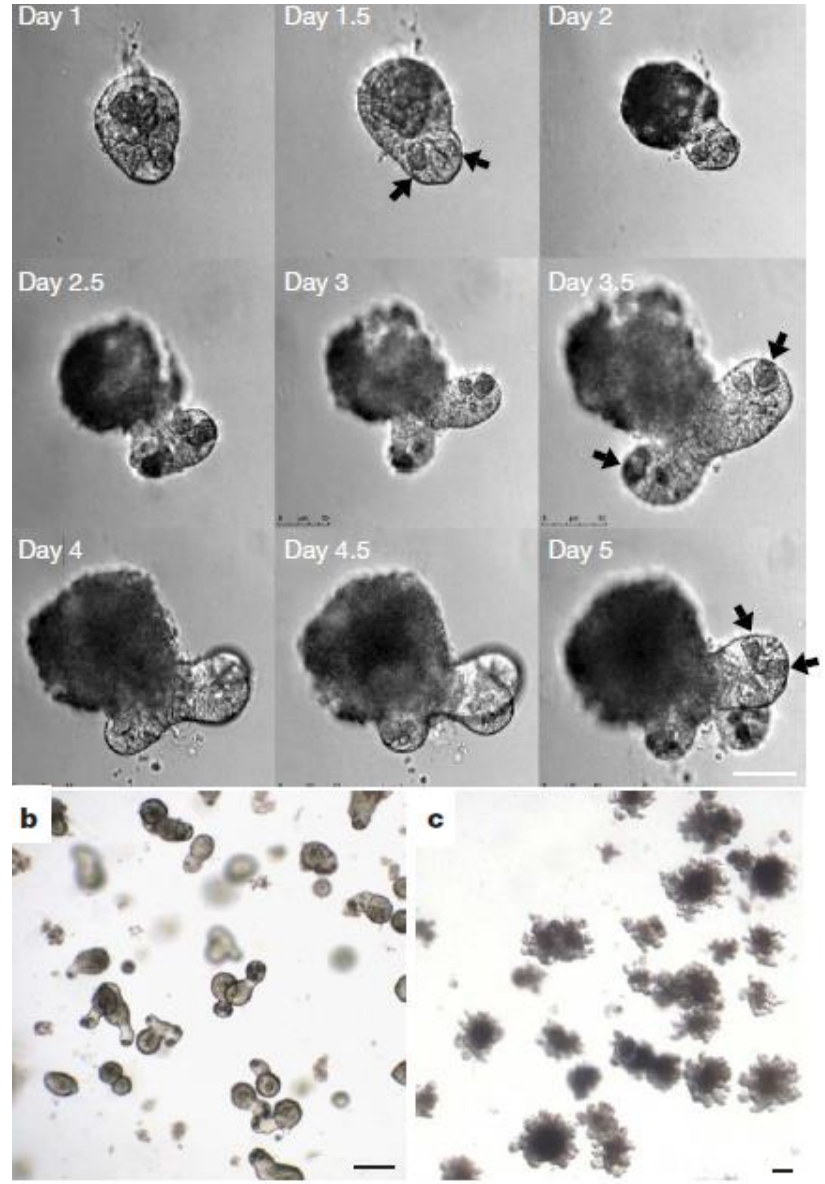
nature Vol 459|14 May 2009|doi:10.1038/nature07935

LETTERS

Single Lgr5 stem cells build crypt–villus structures *in vitro* without a mesenchymal niche

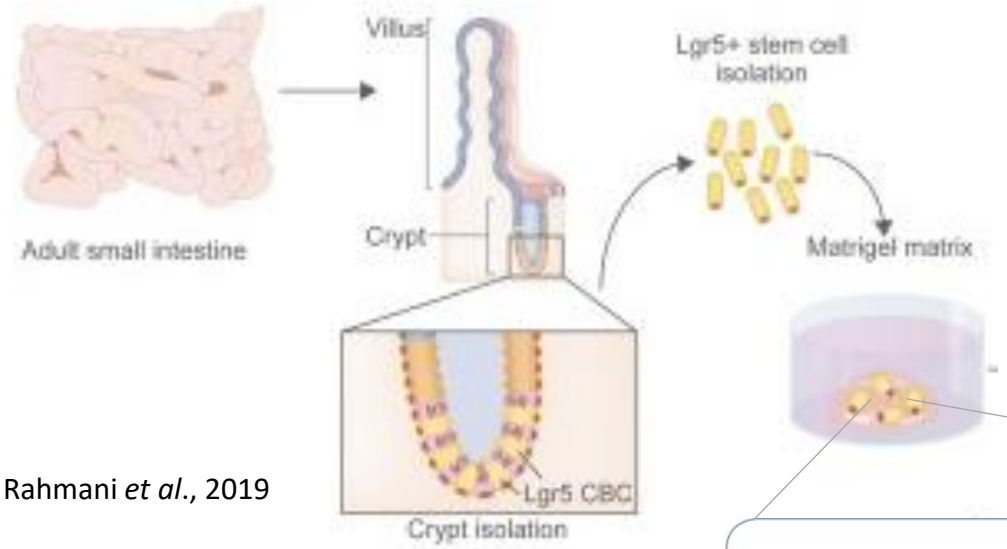
Toshiro Sato[1], Robert G. Vries[1], Hugo J. Snippert[1], Marc van de Wetering[1], Nick Barker[1], Daniel E. Stange[1], Johan H. van Es[1], Arie Abo[2], Pekka Kujala[3], Peter J. Peters[3] & Hans Clevers[1]

The development of the intestinal organoid derived from LGR5+ cells by Hans Clevers' group provided a brand new tool for modeling and studying the pathogenesis of diseases and the therapeutic effects of novel medicines, including bacterial enteritis.
Since then, many studies have been conducted using intestinal organoids.



Day 5 Day 14

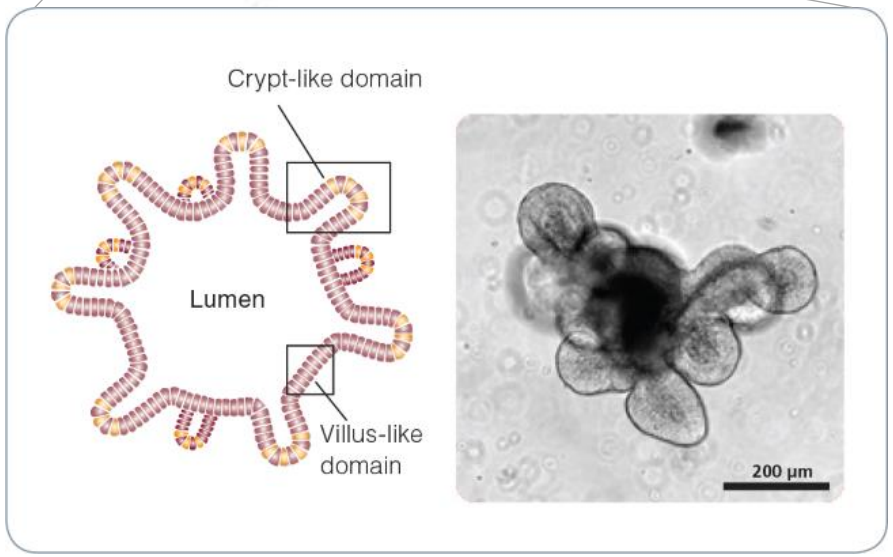
Intestinal organoids: promising and unprecedented new tool



Rahmani *et al*., 2019

Intestinal organoids or mini-gut derive from *ex vivo* culture of intestinal stem cells and are capable of closely replicating the structure and cellular composition of a functional native intestinal epithelium.

The term "enteroids" refer to multilobulated structures with a lumen that develops from intestinal stem cells (cycling crypt base columnar cells and quiescent stem cells) near the bottom of the intestinal crypts.



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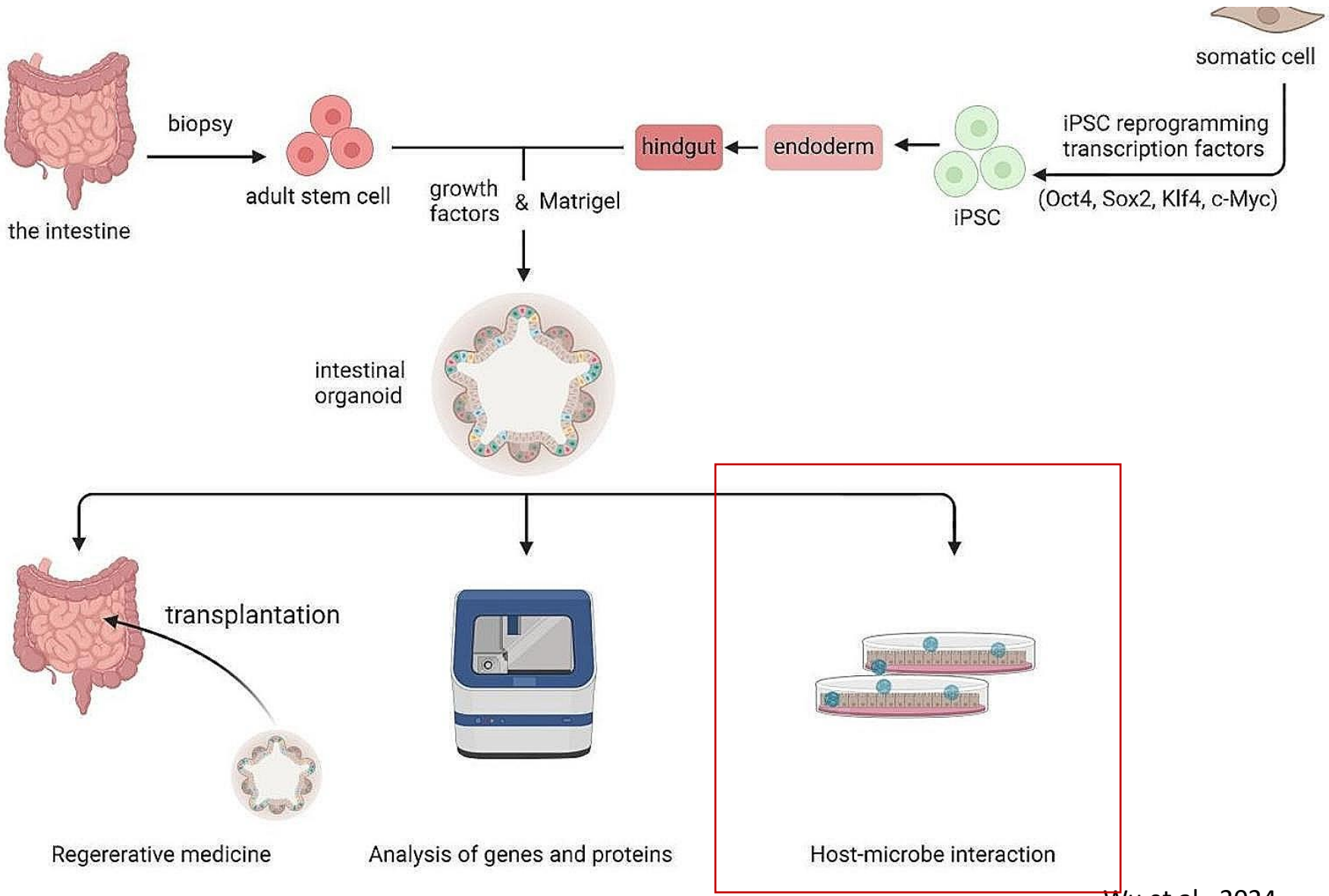
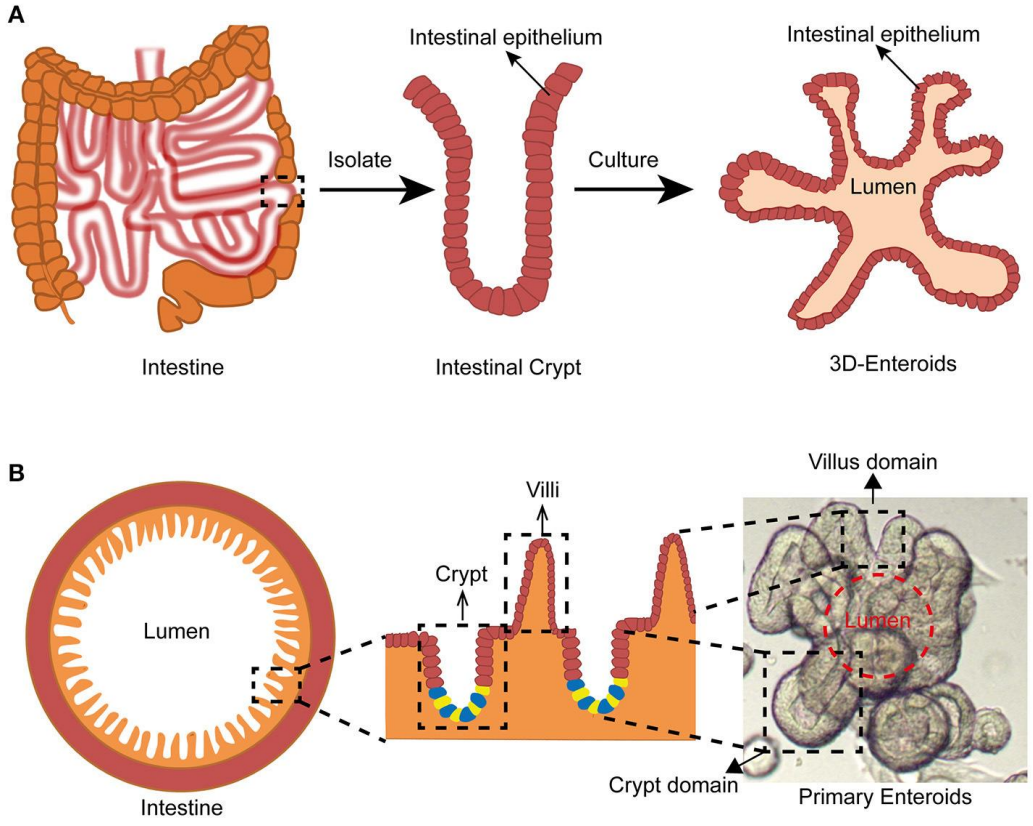


Wu et al., 2024

Enteroid model and its potential application in studying host-pathogen interaction

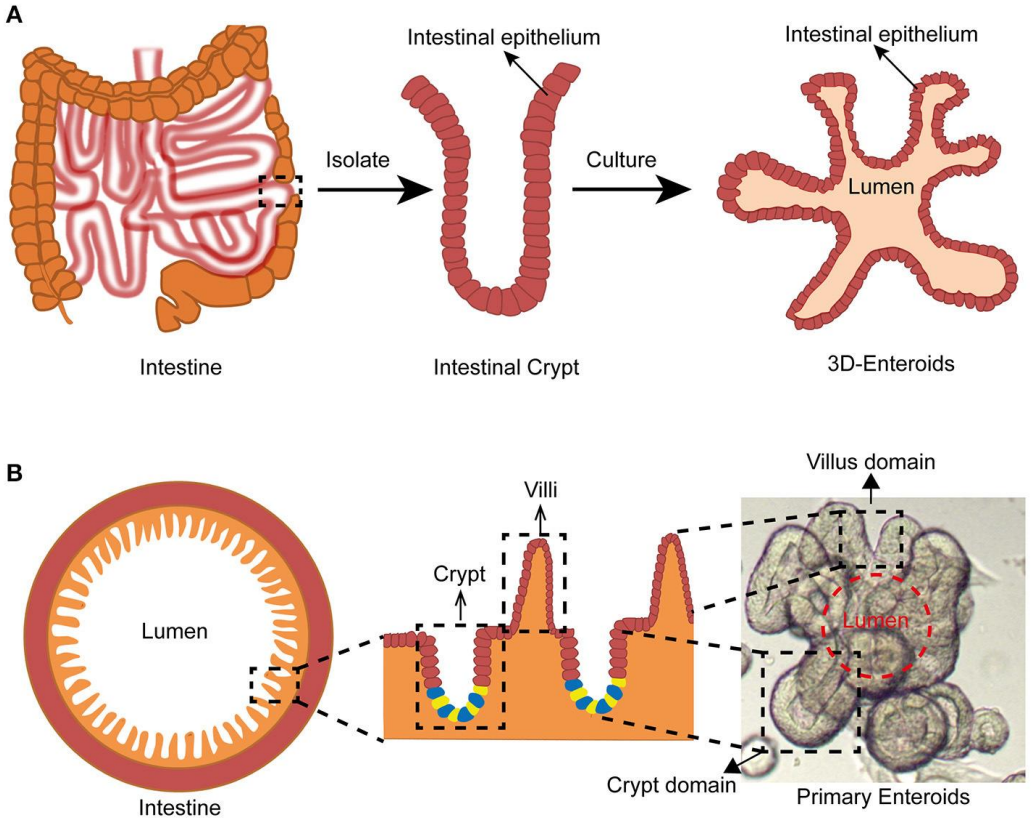


Adapted from: Yin and Zhou *et al*., 2018

ADVANTAGES

- emerging as effective infection models due to their closeness in mimicking the infected tissues/organs (which contains the cell populations and tight junctions normally present in nature) → successfully used to explore bacterial pathogenesis.
- Different bacteria colonize different intestinal segments, and this difference cannot be reflected in previous studies conducted in cell lines. Organoids can be derived from adult stem cells from different segments of the intestine, enabling them to retain specific transcriptional characteristics, epigenetic features, and the expression patterns of immune-related genes that can represent the intestinal segments which they are derived from. This enables organoids to more accurately reflect the biological characteristics, especially the immune response to bacteria of specific intestinal segments.
- Can be ever-expanding, and retain their original organ identity.
- Enteroids also contain luminal layers with crypt and villus domains similar to the real intestine and contain almost all intestinal epithelial cell types including the intestinal stem cells, Paneth cells, Goblet cells, enteroendocrine cells, and enterocytes.

Enteroid model and its potential application in studying host-pathogen interaction

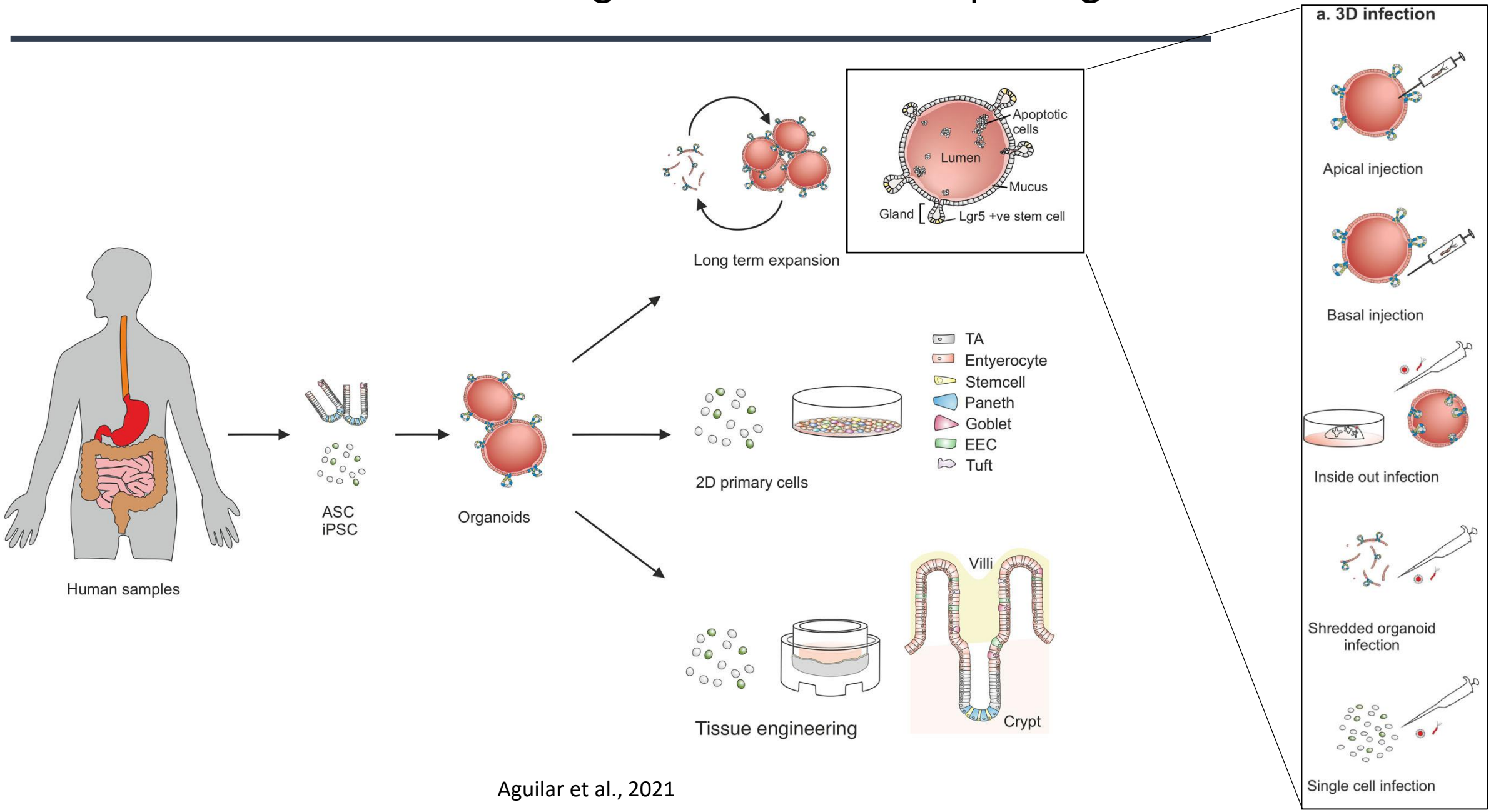


Adapted from: Yin and Zhou *et al.*, 2018

DISADVANTAGES

- Expensive!
- Organoids derived from biopsies, as per characteristic of primary samples, remain highly variable, which is paired with high batch-to-batch variability of organoid media and surrounding ECM (e.g., Matrigel).
- approved guidelines and regulations required.
- the application may not entirely necessitate a physiologically complete model.

Advancements in understanding bacterial enteritis pathogenesis



Aguilar et al., 2021

Experimental models developed to study *Salmonella* infections

TABLE 1 | Salmonella infection models.

	Year	Author	*Salmonella* type	Model
In vitro	2001	Nickerson et al.	*Salmonella* Typhimurium	3D organotypic model based on the human embryonic intestinal epithelial cells (Int-407) (Barrila et al., 2010)
	2006	Zu Bentrup et al.	*Salmonella* Typhimurium	3D organotypic model based on the human colon adenocarcinoma cell line (HT-29 cell line) (Höner Zu Bentrup et al., 2006)
	2008	Isabel Martinez-Argudo and Mark A. Jepson	*Salmonella enterica*	M cell model (Martinez-Argudo and Jepson, 2008)
	2009	Le Blay et al.	*Salmonella* Typhimurium	Colonic fermentation model (Le Blay et al., 2009)
	2012	Tang et al.	Clinical non-typhoid *Salmonella* (NTS) isolates	RAW 264.7 murine macrophage cell line (Tang et al., 2012)
	2014	Dostal et al.	*Salmonella* Typhimurium	Gut fermentation-cell model (Dostal et al., 2014)
	2014	Zhang et al.	*Salmonella* Typhimurium	Crypt-derived mouse intestinal organoids (Zhang K. et al., 2014)
	2015	Forbester et al.	*Salmonella* Typhimurium	Intestinal organoids derived from human induced pluripotent stem cells (hIPSCs) (Forbester et al., 2015)
	2016	Newburg et al.	*Salmonella* Typhimurium	Immature human normal fetal intestinal epithelial cell (H4), mature human metastatic colonic epithelial cell (T84) and human normal colon mucosal epithelial cell (NCM-460) (Newburg et al., 2016)
	2017	Fang et al.	*Salmonella* Typhimurium	HeLa cells, Caco-2 cells, THP-1 cells and LS174T cells (Fang et al., 2017)
ex vivo	1997	Frost et al.	*Salmonella* Typhimurium	Calf ileal epithelium (Frost et al., 1997)
	2004	Haque et al.	*Salmonella* Typhimurium TML	Human intestinal *in vitro* organ culture (IVOC) (Haque et al., 2004)
	2012	Tsilingiri et al.	*Salmonella* Typhimurium	Organ culture model (intestinal mucosa) (Tsilingiri et al., 2012)
	2015	Boyle et al.	*Salmonella* Typhimurium	Perfusion of the isolated rat small intestine (Boyle et al., 2015)
	2016	Newburg et al.	*Salmonella* Typhimurium	Immature human intestinal tissue (Newburg et al., 2016)
In vivo	1973	Giannella et al.	*Salmonela* Typhimurium	The ligated rabbit ileal loop model (Giannella et al., 1973)
	2003	Barthel et al.	*Salmonella* Typhimurium	C57BL/6 mice (Barthel et al., 2003)
	2007	Woo et al.	*Salmonela* Typhimurium	SLC11A1 wild type mice (Woo and Berk, 2007)
	2009	Ren et al.	*Salmonella* Typhimurium	C57BL/6 mice (Ren et al., 2009)
	2011	Mian et al.	*Salmonella* Typhi	Humanized mice (alymphoid RAG-2-/-γc-/- mice engrafted with human leukocytes) (Firoz Mian et al., 2011)
	2012	Özkaya et al.	*Salmonella* Typhimurium	BALB/c mice (Özkaya et al., 2012)
	2012	Mathur et al.	*Salmonella* Typhi	A mouse model (tlr11-/+ mice) (Mathur et al., 2012)
	2014	Zhang et al.	*Salmonella* Typhimurium	Neonate mice (Zhang Y. G. et al., 2014)

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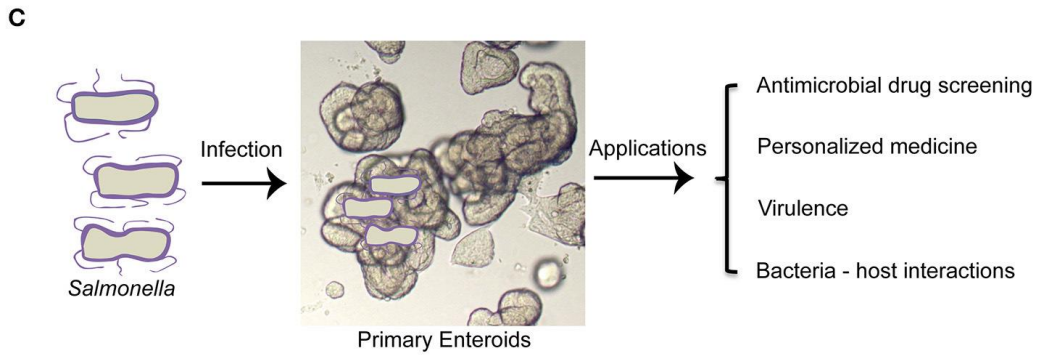
In vitro cell culture lines are relatively easy to maintain and provide a more consistent environmental niche for evaluating bacterial survival and replication than most animal hosts. Genetic manipulations in these cell lines greatly aided the investigation of how *Salmonella* interact with host epithelial and macrophage cells

Animals possess the complex cell types, architectural organizations, and specialized organ structures. More importantly, the intact immune systems of the animals have obvious advantages over all other models and therefore are considered the closest to clinical settings over in vitro cell or ex vivo organ and tissue models.

Organoid and Enteroid Modeling of *Salmonella* Infection



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It has been shown that *Salmonella* quickly attaches and invades the enteroids causing the typical morphologic changes of the host cells during *Salmonella* invasion as well as the disruption of epithelial tight junctions.

https://figshare.scilifelab.se/articles/media/Time-lapse_Movies_for_Geiser_et_al_2021_mBio_Salmonella_enterica_Serovar_Typhimurium_Exploits_Cycling_through_Epithelial_Cells_to_Colonize_Human_and_Murine_Enteroids_/12998570/1?file=25766672