Journal of Pharmaceutical Research International

33(43B): 97-106, 2021; Article no.JPRI.72713
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

A Review on *In vitro* Cell Culture Model for Bacterial Adhesion and Invasion: From Simple Monoculture to Co-Culture Human Intestinal Epithelium Model

Nur Intan Hasbullah[1,2,3], Sharifah Aminah Syed Mohamad[1,2*], Rashidah Iberahim[3], Nor'Aishah Hasan[3], Noorlis Ahmad[3], Low Kheng Oon[4], Nor Azfa Johari[4] and Mohd Nuruddin Abd. Manap[5]

[1]School of Biology, Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia.
[2]Atta-ur-Rahman Institute for Natural Product Discovery, UiTM Puncak Alam Campus, 42300 Puncak Alam, Selangor, Malaysia.
[3]Faculty of Applied Sciences, Universiti Teknologi MARA, Cawangan Negeri Sembilan, Kampus Kuala Pilah, 72000 Kuala Pilah, Negeri Sembilan, Malaysia.
[4]Malaysia Genome Institute, National Institutes of Biotechnology Malaysia, 43000, Kajang, Selangor, Malaysia.
[5]Putra Agrotech Sdn. Bhd. (UPM), 43650 Bandar Baru Bangi, Selangor, Malaysia.

Authors' contributions

This work was carried out in collaboration among all authors. Author NIH wrote the original draft. Author SASM managed supervision and review and editing. Authors RI and NH administered the project. Authors NA and LKO managed the resources. Authors NAJ and MNAM play role in funding acquisition. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i43B32530
Editor(s):
(1) Dr. S. Prabhu, Sri Venkateswara College of Engineering, India.
Reviewers:
(1) Nadjia Benhamed, university of sciences and technology of Oran Mohamed Boudiaf, Algeria.
(2) Aiyelabola Temitayo, Obafemi Awolowo University, Nigeria.
Complete Peer review History: https://www.sdiarticle4.com/review-history/72713

Mini-review Article

Received 15 June 2021
Accepted 20 August 2021
Published 09 September 2021

ABSTRACT

Aim: This paper reviews the different *in vitro* models of human intestinal epithelium that have been utilized for studying the adhesion and invasion properties.
Problem Statement: The cell adhesion and invasion are the key mechanisms of bacterial pathogenicity that determines their possible routes of transmission. Numerous investigations

**Corresponding author: E-mail: sharifah459@uitm.edu.my;*

related to the adhesion and invasion ability of bacterial isolates have been reported on monoculture human intestinal cells. However, the use of monoculture cells has several major disadvantages, such as the inability to reproduce the complex structure that defines the intestine and the inability to accurately predict the mechanism of bacterial adhesion and invasion.
Approach: Co-culture models of human intestine have been developed as an alternative to improve the monoculture epithelial cell for adhesion and invasion studies, which provide more flexibility and overcome some of the limitations
Conclusion: With the use of diverse *in vitro* approach, it could provide thorough information on different ability of bacterial adhesion and invasion and it could help to clarify the intricacy of host-pathogen interactions that underpin bacterial pathogenesis.

Keywords: Human intestinal cell lines; bacterial adhesion; bacterial invasion; monoculture; co-culture Caco-2/HT29-MTX.

1. INTRODUCTION

The intestinal adhesion and invasion of the epithelium are important stages in initiating the bacterial pathogenesis, as the virulent effects of intestinal pathogens rely on their ability to colonize and invade the intestinal mucosa [1-3]. Hence, the entry of bacteria into epithelial cells is essential for its pathogenicity, intracellular replication, spread to other tissues, and cause intestinal disease [4-8]. The ingested bacteria undergo the infection process in both intestinal phagocytic and non-phagocytic cells, which include bacterial adherence, colonisation, invasion, and propagation [9-10].

Due to the scarcity of accurate and specific experimental models, the molecular complexity of host-pathogen interactions in many infectious diseases, especially in humans, remains poorly understood [11]. As a result, a systematic cell culture method for examining the adhesion and invasion capabilities of bacteria is critical for understanding this host-pathogen interaction *in vitro*. Various intestinal epithelium models have been used to investigate the potential for bacterial pathogenicity via adhesion and invasion. The mucosa surface of the intestinal epithelium is a complex environment consisting of several cell types which are enterocytes, goblet cells, Paneth cells and endocrine cells. However, absorptive and goblet cells are the two main constituents of the intestine [12-13]. The Caco-2 cell lines are derived from intestinal absorptive, whereas HT29 and HT29-MTX are known as goblet cells. Caco-2 cells are originated from colon carcinoma that can be differentiated into enterocytes-like cells [14-16]. This monoculture is often used as *in vitro* model for studying cell adhesion and invasion [5,9,17]. However, the use of Caco-2 and other monocultures of epithelial cells has their own disadvantage. As no mammalian system consists of a single cell-type, these monocultures may not accurately depict intestinal physiology *in vivo,* and they do not closely simulate the composition of the normal epithelial layer which contains a variety of cell types [18-20].

Hence, in order to attain better physiological conditions, co-cultivation of two cell lines will therefore provide a model consisting of two different cell types that predominately represented in normal human intestinal epithelium, namely enterocytes and goblet mucus-secreting cells. Thus, this review aims to discuss the establishment of an *in vitro* co-culture cell model completely resembles the small intestinal epithelial layer, based on intestinal enterocytes (Caco-2) and mucus-secreting goblet cells (HT29 and HT29-MTX), to evaluate the adhesion and invasion capabilities of pathogenic and non-pathogenic bacteria.

2. MONOCULTURE OF HUMAN INTESTINAL CELLS FOR ADHERENCE AND INVASION STUDIES

The gut mucosa, which is lined with epithelial cells, is thought to be the most outer defence barrier, preventing microbes and endotoxins from reaching systemic organs and tissues [21]. Thus, studies for adherence and invasion properties of microorganisms have been carried out using single cells, also known as monocultures of human epithelial cell lines such as Caco-2 (non-mucus secreting), HT29 (low mucus secreting) and HT29-MTX (high mucus secreting) (Table 1).

Caco-2 cells are derived from human colon carcinoma and are employed as a model for mature human enterocytes since they can

express protein features of both colonocytes and small intestinal enterocytes immediately after confluence [27,33,43]. This cell line, however, is classified as non-mucus producing cell. Caco-2 cell lines have been extensively utilized as a model to examine *in vitro* adhesion and invasion ability of bacteria such as *Salmonella enterica, Listeria monocytogenes* (*L. monocytogenes*), *Escherichia coli* (*E. coli*), *Campylobacter jejuni* (*C. jejuni*) and probiotics.

Table 1. Studies of bacterial adhesion and invasion using the three most common monoculture of human intestinal cells

Monoculture	Bacterial Strains	Adhesion Study	Invasion Study	References
Caco-2	*Salmonella enterica*	+	+	4,22-27
		+	-	28
		-	+	17,29-31
	Listeria monocytogenes	+	+	23,28,32
		+	-	33
		-	+	31
	Escherichia coli	+	+	24
		+	-	33-34
		-	+	31
	Campylobacter jejuni	-	+	31
	Probiotics	+	-	2,33,35-36
HT29	*Salmonella enterica*	+	+	27
		-	+	30
	Listeria monocytogenes	+	+	32
	Escherichia coli	+	-	37
	Campylobacter jejuni	+	+	38-39
		+	-	40
	Probiotics	+	-	2,35,41
HT29-MTX	*Salmonella enterica*	+	+	27,42
		-	+	30
	Escherichia coli	+	+	42
		+	-	37
	Campylobacter jejuni	+	+	38-39
		+	-	40
	Probiotics	+	-	36, 41

HT29 cells are likewise derived from colon carcinoma, but they are less differentiated than Caco-2 and have a small proportion of mucus secreting cells [27,37,43]. Several studies have been published comparing the adhesion and invasion abilities of HT29 and Caco-2 cell lines. Duary et al. [35] evaluated the adhesion properties of selected indigenous probiotic *Lactobacillus* strains on Caco-2 and HT29 cells. Both cell line showed a similar trend in adhesion property of the test cultures. This finding agrees with Moroni et al. [32] on the adhesion of *L. monocytogenes*. Their findings revealed no significant differences in adhesion between Caco-2 cells and HT29 cells, but it is of interest to note that the level of invasion was higher with HT29 cells as compared to Caco-2 cells. In contrast to the adhesion findings from Duary et al. [35] and Moroni et al. [32], Sharma and Kanwar [2] discovered that the percent of adhesion for lactic acid bacteria isolated from fermented to Caco-2 cells was lower than that of HT29 cells.

Due to the small proportion of mucus secreted by HT29 cells, the treatment of HT29 with methotrexate results in a persistent sub-population of mucus secreting cells (HT29-MTX) that have a differentiated goblet cell-like phenotype and release mucin similar to small and large intestine [27,33]. For this reason, HT29-MTX cells have been chosen in various investigations to study bacterial adherence and invasion. Burkholder et al. [42], for example, demonstrated that *Salmonella* Javiana adhered to HT29-MTX cells at similar levels to *E. coli* but had considerably more invasion than *E. coli*.

In particular, the majority of researchers conclude that the presence of mucus plays an important role as protective component of the normal intestinal epithelium and contributes to the pathogen adherent and invasion. Several studies have been conducted to compare the adhesion and invasion capability between HT29 and HT29-MTX cell lines. Rodrigues et al. [38] developed an experiment in 2018 to test the adhesion and invasion of *C. jejuni* strain towards both HT29 and HT29-MTX. They discovered that the presence of mucus affected the capacity of *C. jejuni* strains to adhere. In 2016, Pilchová et al. [41] demonstrated the potential probiotic effect of *Carnobacterium* strains to attenuate the pathogenesis *L. monocytogenes*. The number of probiotic strains that adhered to HT29-MTX was substantially larger than the number of strains that adhered to HT29. Their report was attentively followed the adherent trend of *C. jejuni,* which showed higher numbers adherent to HT29-MTX cells than the HT29 cells [40]. These results are consistent with those of Alemka et al. [39], who found 10-fold greater levels of *C. jejuni* infection in HT29-MTX than in HT29 cells. On the other hand, intestinal mucus, had no effect on *E. coli* colonization in HT29 and HT29-MTX, according to Kerneis et al. [37]. The level of bacterial adherence to the mucus secreting intestinal cells HT29-MTX also appeared to be higher than attachment to enterocyte-like Caco-2 cells [36].

From literature, there are few studies that comparing the pattern of bacterial adhesion and invasion using all three types of intestinal monoculture that have been discussed in this review. Gagnon et al. [27] studied the suitability of the mucus-secreting HT29-MTX cell model to test adhesion and invasion of *Salmonella* strains and compared with data obtained with the more commonly used Caco-2 and HT29 models. They found that *Salmonella* adhesion and invasion were more effective in HT29-MTX than in non- and low-mucus producing Caco-2 or HT29 cells, respectively. They also suggested that *Salmonella* might potentially permeate the protective mucus layer and subvert the mucus to facilitate invasion. Similarly, in 2019, Li et al. [30] investigated the function of MUC1 (highly expressed mucins in stomach and intestinal tract) during the invasion of *Salmonella* to Caco-2, HT29 and HT29-MTX cell lines. According to the results, Caco-2 and HT29 had lower level of invasion than HT29-MTX cells. They also discovered that, as compared to HT29-MTX, Caco-2 and HT29 cells express comparatively low levels of MUC1. Therefore, these findings prove that mucus aids *Salmonella* adherence and invasion of. The presence of mucus by HT29-MTX cells, in particular, is thought to play crucial function as protective component of the normal intestinal epithelium, enhancing pathogen adherence and invasion.

Since adhesion and invasion are the most important stages in bacterial pathogenesis, an anti-adhesion and anti-invasion therapies are needed as therapeutic strategies or antibiotic therapies to prevent bacterial adhesion/invasion to the host or detachment from the tissues at the early stages of infection [44-46]. For example, designing a synthetic peptide that mimic the structure of pilus protein will inhibit pilus

assembly (pilicides) [47]. This approach is a key strategy for preventing adhesion of *E. coli* and *Salmonella* that used the pili for initiating their virulence factor [48]. Furthermore, the adhesion of pathogenic bacteria could also be disrupted some dietary supplements that acts as receptors analogs [44, 46). Human milk which rich in oligosaccharides and Bovien Muc1 derived from cow milk are proven to inhibit adherent of *E. coli* and *Salmonella* to the Caco-2 cell lines [49-51]. Aside from that, several compounds with anti-invasion properties were discovered to be able to inhibit adhesion and invasion in monoculture Caco-2 cells. Citrus extracts effectively reduced *Salmonella* and *L. monocytogenes* adhesion and invasion to Caco-2, according to Barbosa et al. [23]. Barzelighi et al. [24] investigated whether the presence of azurin reduced *Salmonella* and *E. coli* adhesion and invasion toward Caco-2 cells. Mechesso et al. [4] demonstrated that the presence of ginsenoside Rg3 reduced *S*. Typhimurium adhesion and invasion by two-fold when compared to those lacking Rg3. Similarly, the adherence of *S*. Typhimurium was reduced by 50-70%, and the invasion was inhibited in the presence of methyl gallate [22] and Coenzyme Q0 [25].

In addition, probiotic bacterial strains can be considered as one of the anti-adhesion therapies as they compete with the pathogens for vital growth nutrients [52]. In 2019, Śliżewska and colleagues [28] investigated the competition between probiotics and pathogenic bacteria. They discovered that probiotics reduced the adherence of pathogenic *Salmonella* Typhimurium (*S.* Typhimurium), *Salmonella* Enteritidis (*S*. Enteritidis), and *L. monocytogenes* to Caco-2 cells by up to 60%. The findings are similar to those of Tuo et al. [34], who discovered that probiotic *Lactobacillus* strains prevent *E. coli* from binding to Caco-2 cells. The anti-invasive ability of probiotics strains was also examined on *Salmonella enterica*, *L. monocytogenes*, *E. coli* and *C. jejuni.* The strains were found to have reduced invasive capacity of *S*. Typhimurium [29], *S*. Enteritidis, *L. monocytogenes*, *E. coli* and *C. jejuni* [31] with varying degrees depending on the bacterial species. The ability of probiotics as both anti-adhesion and anti-invasion were also proved to be effective against *S*. Typhimurium and *S*. Enteritidis towards Caco-2 cells [26].

3. CO-CULTURE HUMAN INTESTINAL MODEL FOR ADHERENCE AND INVASION STUDIES

It is undoubted that one single cell line or monoculture does not adequately represent the human intestine. Consistent with the hypothesis that good adherence and invasion especially in the presence of mucus, the goblet cell has been successfully used to co-cultured with Caco-2 cells as an alternate technique to imitate *in vivo* human intestinal physiology. This consideration has led to a method for bridging the gap between simple *in vitro* models and *in vivo* biological process [12,53].

The co-cultures previously proposed in literature for evaluating the mechanism of bacterial adhesion and invasion were obtained by using mucus secreting HT29 subclones which is HT29-MTX to generate a mixed population of enterocytes and mucus secreting cells (Caco-2/HT29-MTX) resembling as closely as possible the intestinal epithelium (Table 2).

Table 2. Caco-2/HT29-MTX co-culture model for bacterial adhesion and invasion

Description of study	References
Identification of potentially effective synbiotics for probiotics adherence towards intestinal mucosa	1
Evaluation on the effect of mucus layer by the presence of *Lactobacillus rhamnosus* and *E. coli*	54
Effect of selected milk and milk protein fractions on the adhesion ability of selected *Lactobacilli*	12
Effect of acid-hydrolyzed milk on the adhesion ability of probiotic strains	55
Effects of prebiotic fructooligosaccharides (FOS) on the adherent ability of *E. coli* (Nissle 1917)	56
Effects of iron concentration on *Salmonella* adhesion, invasion and cellular immune responses	53
Adhesion ability of probiotic, commensal and pathogenic bacterial strains	33

Bacterial adhesion is influenced by surface characteristics, which are influenced by the structure and composition of the cell wall [1,12]. Krausova et al. [1] aimed to identify potentially effective synbiotics by analyzing the adherence of bacterial strains to a Caco-2/HT29-MTX co-culture cell line model that mimicking the intestinal epithelium. They reported high adherence for all strains tested after integrating HT29-MTX cells for mucin synthesis in this model. Several intestinal bacteria have been found to be able to permeate the mucus glycan and use it as a carbon source and attachment site [57-58].

However, their results were in conflict with those obtained by Laparra and Sanz [33], who used similar model and found very low adherence by lactobacilli and bifidobacteria. They differentiated the adhesion pattern of probiotic, commensal and potentially pathogenic bacteria (*E. coli* and *L. monocytogenes*) on both Caco-2 monoculture and co-culture of Caco-2/HT29-MTX. Their findings demonstrated that bacterial adhesion values on Caco-2 monoculture were higher than those in Caco-2/HT29-MTX co-culture, implying that a mucin layer formed by HT29-MTX cells could cover putative recognition components in Caco-2 plasma membrane, rendering them inaccessible to bacteria. Above findings are supported by the fact that the presence of mucus in the model system is significant for estimating intestinal permeability as the mucus acts as a barrier against the absorption of certain compounds [54,59]. The lack of mucus in Caco-2, on the other hand, permits easy access to the cells, leading to an overestimation of their permeability [13]. Consequently, the presence of intestinal mucus may significantly affect adherence, as observed in studies with cell lines that secrete or do not secrete mucus [1].

With the goal of providing an epithelial monolayer covered with mucus that better mimicked the situation *in vivo*, Dostal et al. employed the Caco-2/HT29-MTX co-culture model to explore the interactions of the enteropathogen *S.* Typhimurium through adhesion and invasion towards intestinal cell under different iron concentrations. Under high iron conditions, *Salmonella* adhesion was increased by 8-fold compared to normal iron conditions while invasion was not significantly lowered. On the other hand, in low iron conditions, the invasion of *Salmonella* was significantly increased as compared to normal iron condition. Meanwhile, in 2020, Limage et al. [54] used Caco-2/HT29-MTX co-culture as *in vitro* model of gastrointestinal tract to determine how the mucus layer was affected by the presence of Gram-positive, commensal *Lactobacillus rhamnosus* (*L. rhamnosus*) and Gram-negative, opportunistic *E. coli*. They discovered that, when these bacteria were present and adhered to cells, the secretion of neutral and acidic mucins was altered, and the thickness of the mucus layer was enhanced. In contrast to Caco-2/HT29-MTX, monoculture of Caco-2 cells do not produce mucus and only displayed background levels of staining. However, co-culture of Caco-2/HT29-MT showed more intense staining following adhesion of *L. rhamnosus* and *E. coli* compared to that of unexposed cells.

Similarly, believing that co-cultures of Caco-2/HT29-MTX are better representation of the complex mucosa, Volštátová et al. reported their research in 2016 [55] and 2016 [60] regarding the adherence of probiotic *Lactobacillus* strain. All strains tested in their study *(Lactobacillus plantarum*, *Lactobacillus gasseri* and *Lactobacillus casei*) were all shown to adhere effectively to the co-culture cell lines. Kim et al. [56] also studied the adhering ability of probiotics to the identical Caco-2/ HT29-MTX model used herein since the ability of bacterial strains to adhere to intestinal epithelium cells was regarded as an important selection criterion for probiotics. They discovered that prebiotic fructooligosaccharides reduced the ability of probiotic *E. coli* (Nissle 1917) to adhere to co-cultures Caco-2/ HT29-MTX.

It is of interest to note that until now, no research on the use of co-culture Caco-2 and HT29 cells (low mucus production) to study bacterial adhesion and invasion has been published. Mostly, the co-culture model of Caco-2/HT29 were used to study intestinal permeability of the nanogels and several peptide drugs [61-62]. Therefore, the co-culture of Caco2/HT29-MTX covered with mucus could be considered as the most suitable *in vitro* model of human intestinal epithelium for understanding the bacterial adhesion and invasion, thus the results obtained by using this co-culture model will simulate the human intestine as close as possible.

4. CONCLUSION

Caco-2 cell line has been undoubtedly the most used and accepted *in vitro* intestinal cell model to

study the bacterial adhesion and invasion. However, the mechanism of adhesion and invasion are largely depending on the presence of mucus, an important protective component of the normal intestinal epithelium. Therefore, the HT29-MTX cell model may be more suitable for studying bacterial adhesion and invasion *in vitro* compared to Caco-2 and HT-29 cell models that secrete no or little mucus respectively. The co-culture of Caco-2 cells with the mucus-producing HT29-MTX cell line has been referred as a more predictable experimental cell model than monoculture alone due to the production of mucus that is the most important feature similar to the human intestinal mucosa. Data from this review suggest that the development of co-culture Caco-2/HT29-MTX cell model, with a better physiologically relevant characteristics of mucus layer formation, herein create an excellent *in vitro* system for characterizing cells–pathogens interactions via adhesion and invasion study.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENTS

This work was supported by the FRGS-RACER grant (600-IRMI/FRGS-RACER 5/3 (005/2019). Two of the authors (Hasbullah, N. I. and Hasan, N. A) are registered members under Special Interest Group (SIG), BioMECs, Universiti Teknologi MARA, Cawangan Negeri Sembilan, Kampus Kuala Pilah.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Krausova G, Hynstova I, Svejsti R, Mrvikova I, Kadlec R. Identification of Synbiotics Conducive to Probiotics Adherence to Intestinal Mucosa Using an In Vitro Caco-2 and HT29-MTX Cell Model. Processes. 2021;9(4):569.
2. Sharma S, Kanwar SS. Adherence potential of indigenous lactic acid bacterial isolates obtained from fermented foods of Western Himalayas to intestinal epithelial Caco-2 and HT-29 cell lines. Journal of Food Science and Technology. 2017;54(11):3504–11.
3. Costello CM, Hongpeng J, Shaffiey S, Yu J, Jain NK, Hackam D, et al. Synthetic small intestinal scaffolds for improved studies of intestinal differentiation. Biotechnology and Bioengineering. 2014; 111(6):1222–1232.
4. Mechesso AF, Quah Y, Park SC. Ginsenoside Rg3 reduces the adhesion, invasion, and intracellular survival of Salmonella enterica serovar Typhimurium. J Ginseng Res. 2021;45(1): 75-85.
5. El-hadad D, Desai P, Grassl GA., McClelland, M, Rahav, G, Gal-Mora, O. Differences in Host Cell Invasion and Salmonella Pathogenicity Island 1 Expression. Infection and Immunity. 2016; 84(4):1150-1165.
6. Stecher B, Maier L, Hardt W-D. “Blooming”; in the gut: how dysbiosis might contribute to pathogen evolution. Nat Rev Microbiol. 2013;11:277–284.
7. Thiennimitr P, Winter SE, Bäumler AJ. Salmonella, the host and its microbiota. Curr Opin Microbiol. 2012;5:108–114.
8. Jantsch J, Chikkaballi D, Hensel M. Cellular aspects of immunity to intracellular Salmonella enterica. Immunol Rev. 2011;240:185–195.
9. Birhanu BT, Park NH, Lee SJ, Hossain MA, Park SC. Inhibition of Salmonella Typhimurium adhesion, invasion, and intracellular survival via treatment with methyl gallate alone and in combination with marbofloxacin. Veterinary Research. 2018;49(101).
10. Ribet D, Cossart P. How bacterial pathogens colonize their hosts and invade deeper tissues. Microbes Infect. 2015;17:173–183.

11. Verma S, Senger S, Cherayil BJ, Faherty CS. Spheres of Influence: Insights into Salmonella Pathogenesis from Intestinal Organoids. Microorganisms. 2020;8(4):504.
12. Volstatova T, Havlik J, Potuckova M, Geigerova M. Milk digesta and milk protein fractions influence the adherence of Lactobacillus gasseri R and Lactobacillus casei FMP to human cultured cells. Food Funct. 2016;7(8):3531-3538.
13. Kleiveland CR. Co-cultivation of Caco-2 and HT-29MTX. General Introduction. In: Verhoeckx K. et al. (eds) The Impact of Food Bioactives on Health. 2015;135-139.
14. García-Rodríguez A, Vila L, Cortés C, Hernández A, Marcos, R. Exploring the usefulness of the complex in vitro intestinal epithelial model Caco-2/HT29/Raji-B in nanotoxicology. Food and Chemical Toxicology. 2018;113:162–170.
15. Shah P, Jogani V, Bagchi T, Misra, A. Role of Caco-2 cell monolayers in prediction of intestinal drug absorption. Biotechnol Prog. 2006;22:186–198.
16. Hilgendorf C, Spahn-Langguth H., Regardh CG, Lipka, E, Amidon G, Langguth LP. Caco-2 versus Caco-2/HT29-MTX co-cultured cell lines: permeabilities via diffusion, inside- and outside-directed carrier-mediated transport. J. Pharm. Sci. 2000;89:63–75.
17. Zhou X, Li M, Xu L, Shi C, Shi X. Characterization of Antibiotic Resistance Genes, Plasmids, Biofilm Formation, and In Vitro Invasion Capacity of Salmonella Enteritidis Isolates from Children with Gastroenteritis. Microbial Drug Resistance. 2019.
18. Gibb M, Pradhan SH, Mulenos, MR, Lujan H, Liu J, Ede JD et al. Characterization of a Human In Vitro Intestinal Model for the Hazard Assessment of Nanomaterials Used in Cancer Immunotherapy. Appl. Sci. 2021;11:2113.
19. Araújoa F, Armento B. Towards the characterization of an in vitro triple co-culture intestine cell model for permeability studies. International Journal of Pharmaceutics. 2013;458:128–134.
20. Leonard F, Collnot, EM, Lehr CMA. Three-dimensional coculture of enterocytes, monocytes and dendritic cells to model inflamed intestinal mucosa in vitro. Mol. Pharm. 2010;7:2103–2119.
21. Ghosh SS, Wang J, Yannie PJ, Ghosh S. Intestinal Barrier Dysfunction, LPS Translocation, and Disease Development. Journal of the Endocrine Society. 2020;4(2).
22. Birhanu BT, Lee EB, Lee SJ, Park SC. Targeting Salmonella Typhimurium Invasion and Intracellular Survival Using Pyrogallol. Front Microbiol. 2021;2(12):631426.
23. Barbosa PDPM, Ruviaro AR, Martins IM, Macedo JA, LaPointe G, Macedo GA. Effect of enzymatic treatment of citrus by-products on bacterial growth, adhesion and cytokine production by Caco-2 cells. Food & Function; 2020
24. Barzelighi HM, Esfahani BN, Bakhshi B, Daraei B, Moghim S, Fazeli H. Influence of Heterologously Expressed azurin from Pseudomonas aeruginosa on the Adhesion and Invasion of Pathogenic Bacteria to the Caco-2 Cell Line. Probiotics and Antimicrobial Proteins; 2019.
25. Yang Y, Li J, Yin Y, Guo D, Jin T, Guan N, Shi Y et al. Antibiofilm activity of coenzyme Q0 against Salmonella Typhimurium and its effect on adhesion–invasion and survival–replication. Applied Microbiology and Biotechnology; 2019.
26. Feng J, Wang L, Zhou L, Yang X, Zhao X. Using In Vitro Immunomodulatory Properties of Lactic Acid Bacteria for Selection of Probiotics against Salmonella Infection in Broiler Chicks. PLOS ONE. 2016;11(1).
27. Gagnon M, Berner AZ, Chervet N, Chassard C, Lacroix C. Comparison of the Caco-2, HT-29 and the mucus-secreting HT29-MTX intestinal cell models to investigate Salmonella adhesion and invasion. Journal of Microbiological Methods. 2013;94(3):274-279.
28. Śliżewska K, Chlebicz-Wójcik A, Nowak A. Probiotic Properties of New Lactobacillus Strains Intended to Be Used as Feed Additives for Monogastric Animals. Probiotics and Antimicrobial Proteins. 2021;13:146–162.
29. Ostovan R, Pourmontaseri M, Hosseinzadeh S, Shekarforoush SS. Interaction between the probiotic Bacillus subtilis and Salmonella Typhimurium in Caco-2 cell culture. Iran. J. Microbiol. 2021;13(1):91-97.
30. Li X, Bleumink-Pluym NMC, Luijkx YMCA, Wubbolts, RW, van Putten JPM, Strijbis K. MUC1 is a receptor for the Salmonella SiiE adhesin that enables apical invasion

into enterocytes. PLOS Pathogens. 2019;15(2).
31. Campana R, van Hemert S, Baffone W. Strain-specific probiotic properties of lactic acid bacteria and their interference with human intestinal pathogens invasion. Gut Pathog. 2017;9:12.
32. Moroni O, Kheadr E, Boutin Y, Lacroix C, Fliss I. Inactivation of adhesion and invasion of food-borne Listeria monocytogenes by bacteriocin-producing Bifidobacterium strains of human origin. Applied and environmental microbiology.2006;72(11):6894-6901
33. Laparra JM, Sanz Y. Comparison of in vitro models to study bacterial adhesion to the intestinal epithelium. Letters in Applied Microbiology. 2009;49:695–701.
34. Tuo Y, Song X, Song Y, Liu W, Tang Y, Gao Y et al. Screening probiotics from Lactobacillus strains according to their abilities to inhibit pathogen adhesion and induction of pro-inflammatory cytokine IL-8. Journal of dairy science.2018;101(6);4822-4829.
35. Duary RK, Rajput YS, Batish VK, Grover S. Assessing the adhesion of putative indigenous probiotic lactobacilli to human colonic epithelial cells. The Indian Journal of Medical Research. 2011;134(5):664–671.
36. Bernet MF, Brassart D, Neeser JR, Servin AL. Adhesion of human bifidobacterial strains to cultured human intestinal epithelial cells and inhibition of enteropathogen-cell interactions. Appl Environ Microbiol. 1993;59(12):4121-4128.
37. Kerneis S, Bernet MF, Coconnier MH, Servin AL. Adhesion of human enterotoxigenic Escherichia coli to human mucus secreting HT-29 cell subpopulations in culture. Gut. 1994;35(10):1449–1454.
38. Rodrigues RC, Pocheron AL, Cappelier JM, Tresse O, Haddad N. An adapted in vitro assay to assess Campylobacter jejuni interaction with intestinal epithelial cells: Taking into stimulation with TNFα. Journal of Microbiological Methods. 2018;149:67–72.
39. Alemka A, Clyne M, Shanahan F, Tompkins T, Corcionivoschi N, Bourke B. Probiotic colonization of the adherent mucus layer of HT29MTXE12 cells attenuates Campylobacter jejuni virulence properties. Infection and immunity. 2010; 78(6):2812-2822.
40. Naughton J, Duggan G, Bourke B, Clyne M. Interaction of microbes with mucus and mucins: recent developments. Gut Microbes. 2013;5(1):48-52.
41. Pilchová T, Pilet MF, Cappelier JM, Pazlarová J, Tresse O. Protective Effect of Carnobacterium spp. against Listeria monocytogenes during Host Cell Invasion Using In vitro HT29 Model. Front Cell Infect Microbiol. 2016;26(6):88.
42. Burkholder KM, Fletcher DH, Gileau L, Kandolo, A. Lactic acid bacteria decrease Salmonella enterica Javiana virulence and modulate host inflammation during infection of an intestinal epithelial cell line. Pathogens and Disease. 2019;77.
43. Ouwehand AC, Salminen, S. In vitro adhesion assays for probiotics and their in vivo relevance: a review. Microbial Ecology in Health and Disease. 2009;15(4):175–184.
44. Asadi A, Razavi S, Talebi M, Gholami, M. A review on anti-adhesion therapies of bacterial diseases. Infection; 2018.
45. Krachler AM, Orth K. Targeting the bacteria–host interface: strategies in anti-adhesion therapy. Virulence. 2013;4:284–94.
46. Signoretto C, Canepari P, Stauder M, Vezzulli L, Pruzzo C. Functional foods and strategies contrasting bacterial adhesion. Curr Opin Biotechnol. 2012;23:160–7.
47. Svensson A, Larsson A, Emtenäs H, Hedenström M, Fex T, Hultgren SJ, et al. Design and evaluation of pilicides: potential novel antibacterial agents directed against uropathogenic *Escherichia coli*. Chembiochem. 2001;2:915–8.
48. Chen SL, Hung C-S, Xu J, Reigstad CS, Magrini V, Sabo A, et al. Identification of genes subject to positive selection in uropathogenic strains of *Escherichia coli*: a comparative genomics approach. Proc Natl Acad Sci. 2006;103:5977–82.
49. Parker P, Sando L, Pearson R, Kongsuwan K, Tellam RL, Smith S. Bovine Muc1 inhibits binding of enteric bacteria to Caco-2 cells. Glycoconj J. 2010;27:89–9.
50. Coppa GV, Zampini L, Galeazzi T, Facinelli B, Ferrante L, Capretti R, et al. Human milk oligosaccharides inhibit the adhesion to Caco-2 cells of diarrheal pathogens: *Escherichia coli*, *Vibrio cholerae*, and *Salmonella fyris*. Pediatr Res. 2006;59:377–82.
51. Newburg DS, Ruiz-Palacios GM, Morrow AL. Human milk glycans protect infants

against enteric pathogens. Annu Rev Nutr. 2005;25:37–58.
52. Candela M, Perna F, Carnevali P, Vitali B, Ciati R, Gionchetti P, et al. Interaction of probiotic *Lactobacillus* and Bifidobacterium strains with human intestinal epithelial cells: adhesion properties, competition against enteropathogens and modulation of IL-8 production. Int J Food Microbiol. 2008;125:286–92.
53. Dostal A, Gagnon M, Chassard C, Zimmermann MB, O'Mahony L, Lacroix C. Salmonella Adhesion, Invasion and Cellular Immune Responses Are Differentially Affected by Iron Concentrations in a Combined In Vitro Gut Fermentation-Cell Model. PLOS ONE. 2014;9(3).
54. Limage R, Tako E, Kolba N, Guo Z, García-Rodríguez A, Marques CNH et al. TiO_2 Nanoparticles and Commensal Bacteria Alter Mucus Layer Thickness and Composition in a Gastrointestinal Tract Model. Small. 2020;2000601.
55. Volštátová T, Havlík J, Doskočil I, Geigerová M, Rada V. Effect of Hydrolyzed Milk on The Adhesion of Lactobacilli to Intestinal Cells. Scientia Agriculturae Bohemica. 2015;46(1): 21–25.
56. Kim JK, Shin EC, Park HG. Fructooligosaccharides decreased the ability of probiotic Escherichia coli Nissle 1917 to adhere to co-cultures of human intestinal cell lines. Journal of the Korean Society for Applied Biological Chemistry. 2015;58(1):45–52.
57. Sicard JF, Le Bihan G, Vogeleer P, Jacques M, Harel J. Interactions of Intestinal Bacteria with Components of the Intestinal Mucus. Front Cell Infect Microbiol. 2017;7:387.
58. Guglielmetti S, Tamagnini I, Minuzzo M, Arioli S, Parini C, Comelli E et al. Study of the adhesion of Bifidobacterium bifidum MIMBb75 to human intestinal cell lines. Curr. Microbiol. 2009;59:167–172.
59. Behrens I, Stenberg P, Artursson P, Kissel T. Transport of lipophilic drug molecules in a new mucus-secreting cell culture model based on HT29-MTX cells. Pharm Res. 2001;18:1138–1145.
60. Volštátová T, Havlik J, Potuckova M, Geigerova M. Milk digesta and milk protein fractions influence the adherence of *Lactobacillus gasseri* R and *Lactobacillus casei* FMP to human cultured cells. Food Funct. 2016;7(8):3531-3538.
61. Xavier M, García-Hevia L, Amado IR, Pastrana L, Gonçalves C. In Vitro Intestinal Uptake and Permeability of Fluorescently-Labelled Hyaluronic Acid Nanogels. International Journal of Nanomedicine. 2019;14:9077–9088.
62. Antunes F, Andrade F, Araújo F, Ferreira D, Sarmento, B. Establishment of a triple co-culture in vitro cell models to study intestinal absorption of peptide drugs. European Journal of Pharmaceutics and Biopharmaceutics. 2013;83:427–435.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/72713