Chapter 12
The IPEC-J2 Cell Line

Hans Vergauwen

Abstract IPEC-J2 cells are intestinal porcine enterocytes isolated from the jejunum of a neonatal unsuckled piglet. The IPEC-J2 cell line is unique as it is derived from the small intestine and is neither transformed nor tumorigenic in nature. IPEC-J2 cells mimic the human physiology more closely than any other cell line of non-human origin. Therefore, it is an ideal tool to study epithelial transport, interactions with enteric bacteria, effects of probiotic microorganisms and the effect of nutrients and other feedstuffs on a variety of widely used parameters (e.g. transepithelial electrical resistance (TEER), permeability, metabolic activity) reflecting epithelial functionality.

IPEC-J2 cells undergo in culture a process of spontaneous differentiation that leads to the formation of a polarized monolayer with low or high TEER, depending on the type of serum added to the culture medium, within 1–2 weeks. Porcine serum gives rise to low resistance and normal active transport rates, enabling comparison with the in vivo situation. The high resistance caused by fetal bovine serum can be beneficial to use when investigating compounds having a negative effect on the monolayer permeability or tight junction structures.

There are still many opportunities for exploring the use of these cells as the available research is limited. This chapter will cover the origin, characteristics and methods of the use of IPEC-J2 cells as an in vitro model for several research applications, as well as comparisons between IPEC-J2 cells and other epithelial cell lines.

Keywords IPEC-J2 • Cell line • Small intestinal epithelium • Porcine origin • Transepithelial electrical resistance • Non-transformed • Continuous

12.1 Origin

The intestinal porcine enterocyte cell line (IPEC-J2) is a non-transformed, permanent intestinal cell line (Fig. 12.1). These secondary cells were originally isolated from the jejunal epithelium of a neonatal unsuckled piglet in 1989 by Helen Berschneider

H. Vergauwen (✉)
Department of Veterinary Sciences, Laboratory of Applied Veterinary Morphology, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium
e-mail: hans.vergauwen@uantwerpen.be; chris.vanginneken@uantwerpen.be

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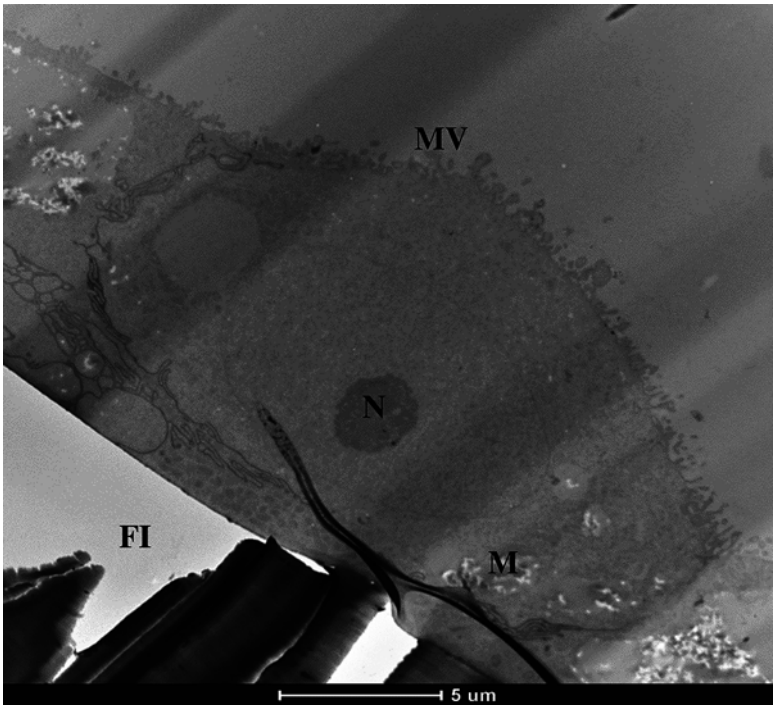

Fig. 12.1 Transmission electron microscopical image of an IPEC-J2 cell grown on a filter insert with fetal bovine serum supplemented to the medium. *M* mucopolysaccharides, *MV* microvilli, *N* nucleus, *FI* filter insert, scale bar: 5 µm

at the University of North Carolina (Berschneider 1989). Given the correct culture conditions, these cells will divide and grow for an infinite number of passages in vitro. To date, they have been cultured continuously for up to 98 passages. IPEC-J2 cells were first used to investigate transepithelial ion transport and enterocyte differentiation (Berschneider 1989). They have already been shown to be a valuable tool in the characterization of epithelial cell interactions with enteric bacteria and viruses providing insight into initial host-pathogen and non-pathogen (e.g. commensal or probiotic) interactions (Brosnahan and Brown 2012).

12.2 Special Features/Morphology/Receptors

The strength of the IPEC-J2 cell line as an in vitro model originates from its morphological and functional similarities with intestinal epithelial cells in vivo. IPEC-J2 cells have microvilli on their apical side and tight junctions sealing neighboring cells together (Schierack et al. 2006). To date, no brush border enzyme

activity has been investigated in IPEC-J2 cells. IPEC-J2 cells form polarized monolayers when cultured on 0.4 μm pore-size filters, with or without a collagen basis. High transepithelial electrical resistance (TEER) values and low active transport rates are obtained when culturing the IPEC-J2 cells in fetal bovine serum (FBS) (Geens and Niewold 2011). These atypically high TEER values can be beneficial to use when investigating compounds having a negative effect on the monolayer permeability or tight junction structures (Vergauwen et al. 2015). Porcine serum (PS) resulted in significantly lower TEER values and higher active transport rates comparable to the in vivo situation (Zakrzewski et al. 2013). When IPEC-J2 cells are grown in 10 % PS they will become taller and smaller in diameter, increasing the tight junction ultrastructure (Zakrzewski et al. 2013).

The protein expression of claudin-1, -3, -4, -5, -7, -8, -12, tricellulin, occludin, E-cadherin and zonula occludens-1 (ZO-1) by IPEC-J2 cells has been confirmed by immunoblotting (Zakrzewski et al. 2013). On the other hand, IPEC-J2 cells do not express claudin-2 and -15 resulting in lower cation selectivity, augmenting the ion permeability of tight junctions compared to the porcine jejunum (Zakrzewski et al. 2013; Schierack et al. 2006).

IPEC-J2 cells express and produce cytokines, defensins, toll-like receptors and mucins. The presence of glycocalyx-bound mucins like Muc1 and Muc3 has been confirmed in IPEC-J2 cells, respectively by RT-PCR and ELISA (Schierack et al. 2006). The expression of the Muc2, the major gel-forming mucin in the human small intestine, was not detectable by RT-PCR (Schierack et al. 2006). Thus, the mucus production by IPEC-J2 cells is not comparable to the in vivo situation. The mucus layer is only a very thin layer that is superimposed on the IPEC-J2 cells when cultured with fetal bovine serum. However, PAS staining showed an increase in mucus production when IPEC-J2 cells were cultured with porcine serum (Zakrzewski et al. 2013).

IPEC-J2 cells express proteins such as MHC I and secrete cytokines like GM-CSF and TNF-α that can establish communication between enterocytes and the immune system. Furthermore, mRNA expression of TLR-1, -2, -3, -4, -5, -6, -8, -9, -10 and IL-1α, -1β, -6, -7, -8, -12A, -12B, -18 has been confirmed in IPEC-J2 cells. TLR-2, -3, -5, -9 and IL-6, -8 proteins were also detected in these cells (Brosnahan and Brown 2012; Arce et al. 2010; Burkey et al. 2009).

IPEC-J2 cells express the F4 fimbrial receptor but not the F18 fimbrial receptor as this has been correlated to older pigs (3–23 weeks). These features make IPEC-J2 cells an interesting in vitro model to investigate the pathogenesis of zoonotic enteric infections that also affect humans.

12.3 Stability/Consistency/Reproducibility of the System

In current research IPEC-J2 cells have been used until passage 98. However, most studies do not mention the passage number. Interlaboratory result evaluation presented a consensus that a confluent IPEC-J2 cell monolayer grown in 5 % fetal

bovine serum corresponds to a TEER value of 1,000 $\Omega \times cm^2$. TEER experiments are usually started at 1,000–3,000 $\Omega \times cm^2$. These values are reached after 4–9 days of culturing on an insert with a semi-permeable membrane (0.4 μm pore size) with or without collagen coating. However, when IPEC-J2 cells are incubated with 10 % PS instead of 5 % FBS TEER values of 400–500 $\Omega \times cm^2$ are obtained. These TEER values are comparable to those found in Caco-2 cell cultures and in vivo.

The amount of cells seeded on the insert depends on the insert size and brand. It has been demonstrated that seeding density can influence TEER values over time, as well as proliferation capacity and functionality. When a 12-well plate insert is seeded with $1–10 \times 10^5$ cells/insert, minimal cell division is needed.

Results obtained from microbial investigations using IPEC-J2 cells show strong correlation with mucosal explants and in vivo. However, various EHEC mutants adhere differently to IPEC-J2 cells and porcine ileal loops. This indicates that different model systems may behave differently (Yin et al. 2009). Nonetheless, the ability of some EHEC mutants to adhere to IPEC-J2 cells and ileal loops was highly correlated.

12.4 Relevance to Human In Vivo Situation

IPEC-J2, IPEC-1 and IPI-2I are three widely used porcine small intestinal cell lines (Arce et al. 2010). IPI-2I cells have been transformed with an SV40 plasmid, whereas IPEC-J2 and IPEC-1 are non-transformed cell lines. IPI-2I cells were isolated from the ileum, IPEC-J2 cells from the jejunum and IPEC-1 cells were isolated from a mixture of ileal and jejunal tissue. IPEC-1 and IPEC-J2 cells were both isolated from one day old piglets, whereas IPI-2I cells were isolated from an adult boar (Berschneider 1989). Furthermore, IPEC-J2 cells, unlike IPI-2I cells, form a polarized monolayer, promoting *S. typhimurium* invasion and replication (Boyen et al. 2009).

Several normal and transformed cell lines are used in food science (Cencic and Langerholc 2010). Caco-2, T84, HT-29, HUTU-80 and SW620 are the most widely used human intestinal cell lines. The majority of human intestinal cell lines is isolated from the colon, and most are tumorigenic. The HUTU-80 cell line is the only widely available small intestinal human cell line. It was derived from the duodenum, but is also cancerous (Brosnahan and Brown 2012).

A drug transport permeability study showed that IPEC-J2 cells form a tighter monolayer compared to Caco-2 cells (Pisal et al. 2008). On the other hand, Caco-2 and T84 cells produce a more pronounced mucus layer compared to IPEC-J2 cells (Navabi et al. 2013). HT29, T84, Caco-2, and SW620 are all negative for IL-2, IL-4, and IFN-γ, while mRNA for IL-1α, IL-8, and TNF-α is variable amongst the human cell lines, but is present in IPEC-J2 cells (Eckmann et al. 1993). Existing cancer-derived cell lines can have limitations such as an altered glycosylation pattern and an aberrant protein expression that define the epithelial character as well as unresponsiveness to hormones or cytokines (Peracaula et al. 2008).

Two major advantages favor IPEC-J2 cells as a model of normal intestinal epithelial cells compared to transformed cell lines: (1) as a permanent cell line they maintain

their differentiated characteristics and exhibit strong similarities to primary intestinal epithelial cells, and (2) IPEC-J2 cells have the advantage of being directly comparable to the experimental animal that is used as an in vivo model for humans. Of all non-primates, the gastrointestinal (GI) tract of pigs is the most appropriate in vivo model as it is similar in size, weight, anatomy and physiology as the human GI system (Deglaire and Moughan 2012).

Due to the close similarity between swine and human intestinal function, studies with IPEC-J2 cells provide valuable insights into the pathogenesis of zoonotic enteric infections that also affect humans (Skjolaas et al. 2006). Extrapolating information can be difficult as in vitro, ex vivo and in vivo cells or tissue can respond differently to environmental stimuli (e.g. diet). Indeed, diet-induced gene expression patterns differ between IPEC-J2 cells and intestinal tissue from preterm and newborn piglets, making interpretation rather difficult (Støy et al. 2013). However, an in vitro model is still a valuable tool to investigate a limited number of factors in a standardized, regulated setting. A comparison of normal diploid IPEC-J2 cells with other transformed or tumorigenic cell lines can give more insight when investigating gene expression and to greater extent the effects of bioactives on intestinal health.

12.5 General Protocol

12.5.1 Culture Conditions

The IPEC-J2 cells are cultured in DMEM/F-12 mix (Dulbecco's Modified Eagle Medium, Ham's F-12 mixture) and supplemented with HEPES, fetal bovine serum (FBS) or porcine serum (PS), insulin/transferrin/selenium (ITS), penicillin/streptomycin and cultivated in a humid environment at 37 °C with 5 % CO_2. The IPEC-J2 cells are usually grown for 1–2 weeks before initiating an experiment. When studying TEER and permeability, IPEC-J2 cells are commonly seeded (1×10^5 cells/well) at confluence in a 'Boyden chamber' insert (upper chamber, apical) on a polyethylene terephthalate (PET) membrane (1.12 cm^2, pore size 0.4 μm) in a 12-well plate (lower chamber, basolateral). Cells are seeded (1×10^5/well) in a 12-well plate (flat bottom) to investigate the intracellular oxidative stress and wound healing capacity. Cells are seeded (0.5×10^4 cells/well) in a 96-well plate (flat bottom) to assess viability. The IPEC-J2 cell line is an easy to use and robust cell line, exhibiting structural and functional differentiation pattern characteristics of mature enterocytes (Geens and Niewold 2011).

12.5.2 Experimental Readout

An increasing number of studies use IPEC-J2 cells to investigate interactions of various animal and human pathogens, including *Salmonella enterica* and pathogenic *Escherichia coli* (Boyen et al. 2009; Veldhuizen et al. 2009). Pathogenic permeation

(e.g. *E. coli*) is usually presented as colony forming units (CFU). IPEC-J2 cells have also been employed as an initial screening tool for adhesiveness and anti-inflammatory properties of potential probiotic microorganisms. IPEC-J2 cells were also used to investigate the effect of prebiotics on the adhesion of probiotic bacterial strains to these cells. Addition of 200 mM calcium has been shown to increase adhesion (Marcinakova et al. 2010), while magnesium and zinc ions had no influence (Larsen et al. 2007). Innate immune responses (e.g. increase in porcine β-defensin 1 and 2 gene expression) in relation to environmental stimuli (e.g. diet or infection) are investigated with relevance for human and porcine intestinal diseases, specifically in newborns (Schierack et al. 2006; Burkey et al. 2009; Veldhuizen et al. 2009). Numerous studies used IPEC-J2 cells to investigate feedstuffs and antioxidants in relation to inflammation, intestinal permeability and wound healing capacity (Hermes et al. 2011; Ma et al. 2012; Pan et al. 2013; Vergauwen et al. 2015).

Permeability is expressed using TEER, either presented as absolute values or as percentages of control or time point (TEERtx/TEERt0). The net value of the TEER ($\Omega \times cm^2$) needs to be corrected for background resistance by subtracting the contribution of the cell-free filter and the medium (80–150 $\Omega \times cm^2$). Alternatively fluorescein isothiocyanate (FITC)-dextran of 4 kDa (FD-4) permeability can be used to indicate monolayer integrity. FD-4 permeability results are presented as a percentage of control or as absolute quantities or concentrations (e.g. picomoles).

Viability and cytotoxicity are most commonly analyzed using the neutral red method, lactate dehydrogenase release or the MTT reduction assay and presented as percentages of control or absorbance values (Table 12.1).

12.5.3 Sample Preparation

It is important only to incubate sterile filtered samples on the IPEC-J2 cells. Otherwise, interpretation of the results will be ambiguous as the effect cannot be contributed to an impurity, a pathogen or the agent of interest.

Samples that are not readily dissolved in water can be dissolved in ethanol or DMSO. The effect of different concentrations of ethanol or DMSO for either 1 or 18 h on the viability of IPEC-J2 cells was investigated (Fig. 12.2). Results show that short and long term incubation do not favor DMSO or ethanol at concentrations below 1 %. DMSO is favored for long-term incubation at concentrations above 1 %. However, concentrations exceeding 1 % are not recommended. Furthermore, it is always important to minimize the concentration of DMSO or ethanol when solubilizing a compound.

12.6 Conclusion

In summary, the IPEC-J2 cell model provides a perfect tool to investigate intestinal epithelial function. This porcine cell line is closely related to the human in vivo situation and is not cancerous compared to other human (small) intestinal cell lines.

Table 12.1 Experimental read-out of specific assays applicable to the IPEC-J2 cell model

Application	Feedstuff/component/toxin/bacteria/virus	Read-out	Positive/negative controls	References
Barrier/transport	FITC-labelled soybean protein P34	Mean fluorescence intensity (MFI)	Negative control: Incubation at 4 °C → no endocytosis, only binding	Sewekow et al. (2012)
Inflammation	Feedstuffs (wheat bran, casein glycomacropeptide, mannan-oligosaccharides, locust bean and *Aspergillus oryzae* fermentation extract)	Relative abundance (%) (RT-qPCR)	Positive control: sterile PBS, no feedstuffs	Hermes et al. (2011)
Proliferation	Possible toxic potential of Cry1Ab protein, commonly expressed in GM-maize	WST-1 conversion (OD_{450nm})	Negative control: No serum	Bondzio et al. (2013)
Adhesion/translocation	Effect of feedstuffs on the numbers of adhered *E. coli* K88 per well	Detection time (h)	Negative control: PBS	Hermes et al. (2011)
Cross-talk	Gut-mediated changes in gene expression of hepatic CYP enzymes by LPS	Relative CYP gene expression	Negative control: No LPS	Paszti-Gere et al. (2014)
Toxicity/viability	*Fusarium* toxin deoxynivalenol (DON)	Lactate dehydrogenase release (U/L)	Vehicle control: 0.5 % ethanol	Awad et al. (2012)
Wound healing assay	Sodium butyrate (SB)	Wound size (µm)	Negative control: No SB	Ma et al. (2012)
Intracellular oxidative stress	Trolox, ascorbic acid and glutathione monoethyl ester	Mean fluorescence intensity (MFI) using CM-H_2DCFDA	Negative control: No antioxidant, no oxidant	Vergauwen et al. (2015)
Intestinal permeability (indirect method)	Soybean agglutinin (SBA)	Alkaline phosphatase (IU/L)	Negative control: No SBA	Pan et al. (2013)

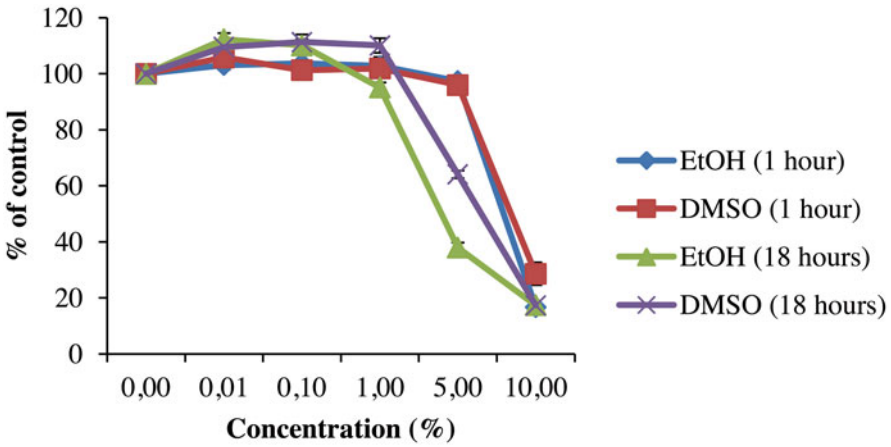

Fig. 12.2 Effect of ethanol and DMSO on the IPEC-J2 cell viability. IPEC-J2 cells were incubated with different concentrations of ethanol (EtOH) and DMSO for 1 or 18 h before assessing the viability using the neutral red method. Results are presented as means ± S.E.M., n = 12

The tumorigenic nature of the human intestinal cell lines can influence gene expression, and transformed cell lines are usually more resistant to stress or cytotoxic insults. This will result in ambiguous information and an underestimation of cytotoxic compounds. Furthermore, IPEC-J2 cell monolayers are ready for experimentation after 1–2 weeks which is a lot faster compared to the 21-day culturing time of Caco-2 cells. In conclusion, IPEC-J2 cells are an ideal small intestinal enterocyte model to study effects of food bioactives in the gut prior to in vivo evaluation.

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