Alternative Models to Study the Intestinal Barrier Function of Piglets

Maartje De Vos, Véronique Huygelen, Christophe Casteleyn, Steven Van Cruchten and Chris Van Ginneken

The intestinal epithelial barrier is anatomically composed of a single layer of enterocytes, joined by junctional complexes that comprise the zonula occludens, zonula adherens and macula adherens. On the one hand, the epithelial layer seals the intestinal tract, so that it forms a robust barrier against the passage of luminal pathogens, toxins and other antigens. On the other hand, the barrier needs to be permeable to facilitate the uptake of nutrients. Weaning young animals, such as piglets, causes mucosal damage and alterations in tight junction integrity, which compromise the small intestinal barrier. Hence, weaning is associated with gastrointestinal disorders such as diarrhoea, and the impaired barrier increases the susceptibility to disease.

Nutrition plays a crucial role in maintaining the intestinal barrier function. Therefore, researchers have investigated the effects of several dietary components (e.g. spray-dried plasma, natural anti-oxidants) in weaned piglets. Various techniques are available to study gut permeability, which all have advantages and shortcomings. The majority of the reported studies in swine use *ex vivo* models. In these terminal experiments, the barrier function of the gut is examined in intestinal segments via, for example, Ussing chambers and everted sac models. These techniques use different marker probes (e.g. ovalbumin and mannitol), that are placed at the mucosal site, so the transcellular and paracellular passage of the marker probes can be determined at the serosal sites.

In our opinion, alternative methods that do not require the sacrificing of animals should be considered. Several alternative techniques are available, even for pigs themselves, which include *in vivo* non-invasive tests and *in vitro* cell models. Intestinal permeability can be determined by measuring orally-administered marker probes, which are recovered in the urine and/or blood. The most frequently used probes are non-metabolised sugars that are not degraded by digestive enzymes, but are fermented by colonic bacteria. They therefore provide information on small intestinal permeability. Differential sugar absorption tests (e.g. with lactulose as a paracellular marker and mannitol as transcellular marker) are widely used in human studies, whereas their use in pigs is surprisingly limited. This technique has its limitations: urine collection in pigs is not an easy task, the presence of food-derived sugars in the urine/blood may interfere, and sugar quantification is expensive. Despite these limitations, the major advantage of this technique is its potential for multiple measurements in the same animal over time. Moreover, the use of multiple sugar substance tests, instead of single test substances, is less influenced by premucosal factors (gastric emptying, intestinal transit time, and bacterial degradation), and by postmucosal factors (metabolism, completeness of urinary collection, and renal function). The ratio of the urinary recovery of the two sugars provides information about the intestinal barrier function. The assumptions are that both the probes are affected by the premucosal and postmucosal factors to a similar extent, and that their ratio is not disturbed by these factors.

Besides sugars, chromium-labelled ethylenediamine tetra-acetic acid (\(^{51}\)CrEDTA) and polyethylene glycol (PEG) can be used as probes. The \(^{51}\)CrEDTA test, employing a single probe, has one major disadvantage: although the radiation dose is low, it exposes the animal to radiation and should be avoided in multiple permeability analyses. As this probe is resistant to bacterial degradation in the colon, it must also be used with caution, in combination with a monosaccharide. In addition, collection times must be short, in order to provide permeability information on the small intestine. PEG probes of different sizes can be used, the analysis of which has been described as 'rapid and simple'. However, the permeation rates of PEG probes are at least 20 times higher than those of monosaccharides, probably due to the lipid solubility of PEG. Therefore, the use of the non-metabolised, non-degradable probes (PEG and \(^{51}\)CrEDTA) provides ‘whole gut’ permeability information, whilst sugars afford the opportunity to provide site-specific information about gut permeability, based upon their differences in digestibility and degradation.
Porcine cell culture models functionally resemble the in vivo situation, and therefore offer a suitable alternative to animal testing. Of particular interest for studying the absorptive mechanisms and intestinal permeability, is a model that only contains absorptive cells. Various porcine intestinal cell lines (CLAB, IPEC-1, IPEC-J2, IPI-21 and PSI-1) are available, but some of them are more appropriate for measuring intestinal permeability. A reliable method to test for monolayer formation and the presence of tight junctions, is the measurement of trans-epithelial electrical resistance (TEER). It is important to point out that the CLAB cell line, an adult mucin-secreting enterocyte-like cell line, fails to generate TEER. In contrast, the PSI-1 cell line generates very high TEER values, up to 7000Ω.cm². This makes the CLAB cell line a good choice for paracellular transport assays, whereas the PSI-1 cell model is more suitable for active transport studies. The majority of in vitro studies, however, utilise the IPEC-1 and IPEC-J2 cell lines. Both these cell lines are derived from the small intestine of a neonatal piglet, and are known to retain most of their original epithelial nature. The two cell lines form tight junctions, express tight junction proteins (e.g. claudin and occludin), and generate TEER values. In addition, they both form microvilli at their apical surfaces. Both IPEC-1 and IPEC-J2 show major similarities with the tissue of origin, which makes it possible to extrapolate the results of the in vitro trials to the in vivo situation. However, in vitro models with only absorptive cells do not reflect the total complex physiology of the porcine gut. Therefore, a number of other cell types, including immune cells, are available for use in co-culture experiments. Despite their limitations, the use of cell lines offers many advantages. Their use is relatively inexpensive, working with cell lines is relatively easy, and large-scale (screening) experiments are possible under controlled conditions.

In conclusion, there are various methods for use in studies on the intestinal barrier function of piglets, and this can assist in the search for alternatives, in order to reduce and/or replace the number of piglets used.

Author for correspondence:
Professor Chris Van Ginneken
Laboratory of Applied Veterinary Morphology
Department of Veterinary Sciences
Faculty of Pharmaceutical, Biomedical and Veterinary Sciences
University of Antwerp
2610 Wilrijk
Belgium
E-mail: chris.vanginneken@ua.ac.be

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