Chapter 24
Ussing Chamber

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Abstract The Ussing chamber system is named after the Danish zoologist Hans Ussing, who invented the device in the 1950s to measure the short-circuit current as an indicator of net ion transport taking place across frog skin (Ussing and Zerahn, Acta Physiol Scand 23:110–127, 1951). Ussing chambers are increasingly being used to measure ion transport in native tissue, like gut mucosa, and in a monolayer of cells grown on permeable supports. However, the Ussing chamber system is, to date, not often applied for the investigation of the impact of food bioactives (proteins, sugars, lipids) on health.

An Ussing system is generally comprised of a chamber and a perfusion system, and if needed, an amplifier and data acquisition system. The heart of the system lies in the chamber with the other components performing supporting roles. The classic chamber design is still in wide use today. However, several newer designs are now available that optimize for convenience and for diffusion- or electrophysiology-based measurements. A well designed Ussing chamber supports an epithelia membrane or cell monolayer in such a way that each side of the membrane is isolated and faces a separate chamber-half. The chambers are then filled with a physiologically relevant solution, such as Ringer's solution. This configuration allows the researcher to make unique chemical and electrical adjustments to either side of the membrane with complete control. The Ussing chamber technique has its strengths and limitations, which will be explained in more detail in this chapter.

Keywords Ex vivo • Intestine • Intestinal barrier • Intestinal transport • Ussing chamber

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24.1 The Ussing Chamber

A commercially available 'classic' Ussing chamber system (Fig. 24.1) is machined from solid acrylic into two halves and has vertical and horizontal ports in each half for connection to the circulation system and for making electrical connections. The face of one chamber-half is imbedded with sharp stainless-steel pins which mate with corresponding holes in the other chamber-half face. These pins allow puncturing and positioning of an epithelium membrane within the chamber. Each chamber-half also has a separate air/gas inlet to drive the circulation system. Gas, commonly a 95 % O_2/5 % CO_2 mixture, is forced under low pressure into these inlets and allows contact with the buffer solution. The rising bubbles drive circulation and oxygenate the buffer. Each chamber-half also has an inlet or outlet port for access to the water jacket. Water at the desired temperature is pumped through the jacket and allowing thermoregulation of the perfusion solutions. Each chamber is supplied with 2 Ag-AgCl pellet electrodes (for voltage) and 2 Ag wire electrodes (for current). Electrodes are connected into the chamber by means of an agar salt bridge.

One of the major limitations of the classic Ussing chamber model is that it does not allow simultaneous preparation and analyses of a large set of segments of epithelial tissues thereby having a relatively low throughput. The original Ussing chamber system incorporates the intestinal tissue segments in a vertical position, whereas other systems also allow horizontal placement (e.g. TNO's InTESTine™ system or Warner Instruments' NaviCyte™). This horizontal setup can be used for transport or toxicology studies using cells or tissue exposed to liquids and semi-solid compounds, such as digested food samples, while the basolateral surface remains exposed to the medium.

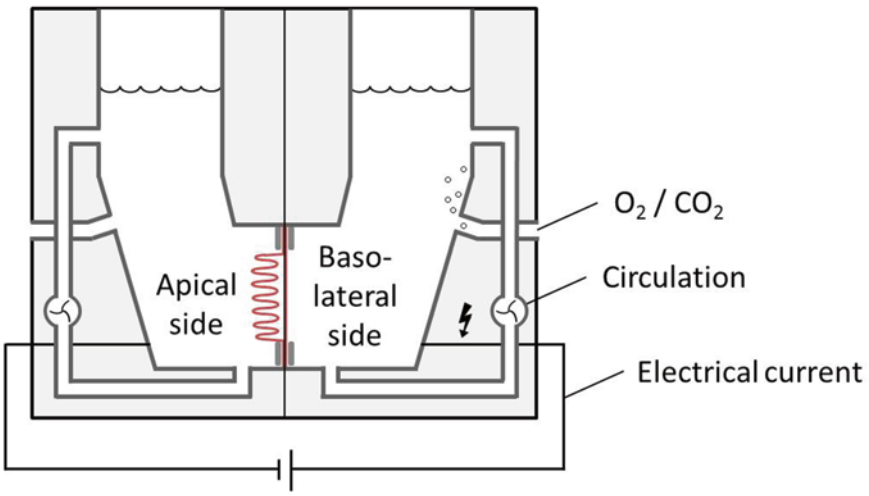

Fig. 24.1 Schematic representation of a small piece of intestinal epithelial tissue mounted in the Ussing chamber

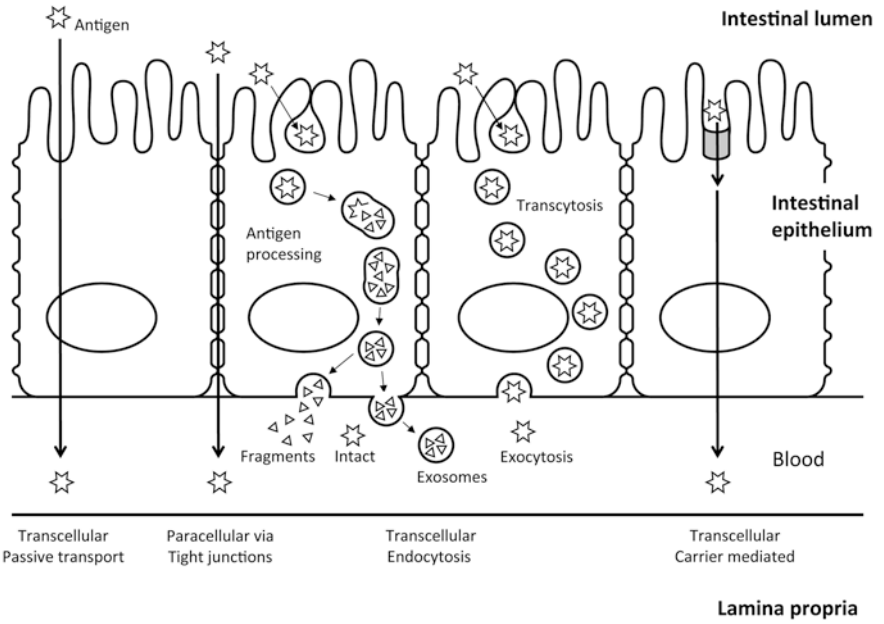

Fig. 24.2 Different transport routes across the intestinal wall (Reitsma et al. 2014, copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission)

Further development of the Ussing chamber system has led to various multichannel systems capable of supporting up to 24 independent chambers, all operating in parallel. TNO recently developed a medium throughput system (InTESTine™) in which up to 96 ex vivo intestinal tissue segments can be used per system per day to investigate intestinal absorption (Westerhout et al. 2014).

Food compounds can be transported across the intestinal barrier by different transport mechanisms (Fig. 24.2). The transport route and velocity of absorption of these compounds are influenced by different factors that are related to physiological factors of the tissue (e.g. composition and thickness of the mucus layer, disease state or membrane permeability, intestinal pH, concentration bile acids, composition of pancreatic juice, surface area, enzyme activity, lipid and protein content of the enterocyte membrane and the amount of Peyer's patches in the tissue), physico-chemical factors of the compound (e.g. water solubility, molecular weight, aggregation state, charge, H-bonding capacity, and hydrophobicity) and formulation factors (e.g. food matrix) (Deferme et al. 2008).

In general compounds are transported via one of the following mechanisms:

(1) Paracellular (e.g. small hydrophilic and polar compounds)
(2) Transcellular

- passive transport (lipophilic compounds)
- endocytosis (e.g. proteins and small nucleotides)
- carrier mediated transport (e.g. glucose and amino acids)

(3) Cell mediated transport

In contrast to single cell-culture models, such as the epithelial Caco-2 cell model, intestinal epithelium consists of absorptive enterocytes and other cells such as goblet cells, endocrine cells, and M cells, with the mucus secreting goblet cells representing the second most frequent cell type, ranging from 10 % in the small intestine and 24 % in the distal colon (Madara and Trier 1987). The transport of food compounds across the intestinal barrier can also be facilitated by the other cell types present in the lamina propria (Fig. 24.3), including M cells, Peyer's patches, dendritic cells and macrophages.

As the Ussing chamber technique uses intestinal tissue segments, they still contain the morphological and physiological features of the intestine, including interplay of many complex processes, such as interaction between the multicellular environment (Rozehnal et al. 2012). Using ex vivo intestinal tissue segments gives a better representation of the complex in vivo morphology (multicellular conglomeration, presence of the mucus layer) and thereby a better representation of the various possible processes involved in the in vivo situation. Furthermore, ex vivo intestinal tissue segments from the different regions of the small and large intestine allow the investigation of regional absorption and immune responses, which is not possible when using single cell-culture models.

The major limitation of using ex vivo intestinal tissue segments is the limited availability of (healthy) human tissue. Therefore, animal tissue is often used. Porcine intestinal tissue could possibly be used as alternative based on its high similarity with that from humans (Patterson et al. 2008; Walters et al. 2011; Groenen et al. 2012). When using human tissue, often a small segment of non-diseased biopsy is

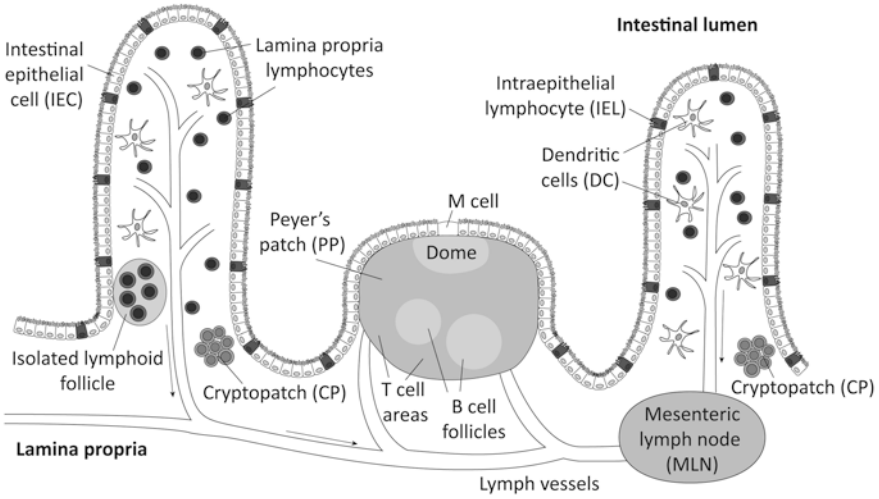

Fig. 24.3 The intestine at a cellular level (Reitsma et al. 2014, copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission)

used, which could be more-or-less influenced by the adjacent diseased tissue. However, besides the effects of the possible side effects of diseased tissue and/or historic drug treatment, the inter-individual variability among humans or any other non in-bred animal model also in a healthy stage is high, making it difficult to directly compare results.

Another major limitation of using ex vivo intestinal tissue segments is the limited viability of the tissue. Previous studies by Haslam et al. (2011), Rozehnal et al. (2012) and Sjöberg et al. (2013) have shown that the intestinal tissue remains viable only for up to 150 min (on average 120 min), which is long enough to study absorption and/or secretion of satiety hormones. For the investigation of the impact of food bioactives on other health effects 2 h may be too short for any clear response, such as release of cytokines, reversible disruption of tight junctions, extensive metabolism. However, changes in mRNA levels within 2 h should be measurable by using reverse transcription polymerase chain reactions (RT-PCR).

24.2 General Protocol

Human intestinal tissue segments are often obtained via biopsies or by surgical resection, whereas intestinal tissue segments from animals are often obtained from section of sacrificed animals. Collect the intestine, directly after opening the abdominal cavity, in a cold Krebs' Ringer Bicarbonate buffer (pH 7.4), supplemented with 25 mM HEPES (KRB/HEPES), which is bubbled with a O_2/CO_2 (95/5 %) gas mixture, prior to use to prevent ischemia (e.g. during transport). The intestine can be divided in different segments; duodenum can be found directly after the stomach and is approx. 20 cm long, followed by the jejunum (approx. 1.0 m), ileum (approx. 1.6 m) and after the cecum you will find the colon (approx. 1.1 m) (DeSesso et al. 2001).

Upon arrival in the laboratory, flush and rinse the intestinal tissue segments carefully with ice cold physiologically relevant buffer, such as KRB/HEPES buffer, to remove luminal debris and put in a beaker with KRB/HEPES on ice. The tissue segments are then opened longitudinally next to the juncture to the peritoneum and the serosa and muscle layers are removed (stripping) while the tissue is submerged in chilled and oxygenated KRB/HEPES buffer. Removal of muscle layers is either performed using scissors keeping the feather scissor at an angle towards the muscles, or using a pair of curved tweezers to gently strip off the muscle layers while holding the rim of the tissue segment with a Kocher. After the muscle layer is gently removed, the tissue is cut clean from fat, connective tissue and parts of the submucosa to reach the blood vessel level. Depending on the system, intestinal tissue segments with a surface area of 0.1–1.8 cm^2 are prepared using for instance a punch. Keep the tissue and segments in cold buffer during the whole procedure. Stripped intestinal segment is then mounted in the Ussing chamber system (Fig. 24.4). Since epithelial tissue is polarized, it contains an apical (mucosal) and basolateral (serosal) side, so pay attention on how to mount the tissue in the system.

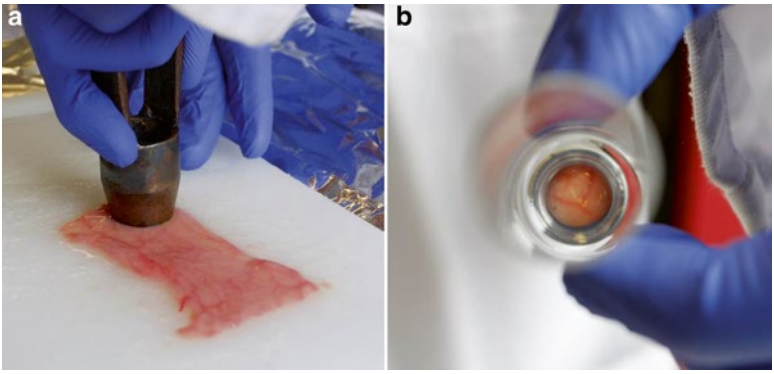

Fig. 24.4 (**a**) Photograph of obtaining a small segment from stripped porcine intestinal tissue by using a hollow punch. (**b**) The smaller segments are then mounted in the InTESTine™ system

Both chamber halves are then filled with ice cold physiologically relevant buffer, such as KRB/Hepes buffer. The basolateral KRB/HEPES buffer is sometimes supplemented with 0.1 % bovine serum albumin (BSA) to enhance intestinal transport. The solution is then oxygenated (95 % O_2:5 % CO_2), circulated, and warmed to 37 °C. Once at 37 °C, carefully flush the mucus layer from the segments and replace the apical buffer by fresh, pre-warmed buffer containing the compound of interest, to investigate the permeability. In most cases compounds are labelled radioactively (e.g. ^{14}C) or with a fluorescent label. During the permeability studies small (100 µl) samples are taken over time from the basolateral and apical compartment for up to 150 min. After 150 min, the viability of the tissue and the integrity of the intestinal barrier starts to decrease (Sjöberg et al. 2013). If desired, the basolateral and apical buffer can be supplemented with fresh, pre-warmed, blank buffer, to complement for the samples taken. Usually, an apparent permeability value (Papp value, unit is cm/s) is calculated according to Eq. (24.1).

$$Papp = \frac{dQ / dt}{SA * C_{Api,0}} \tag{24.1}$$

Here, dQ/dt (in disintegrations per minute (dpm)/s or mg/s) indicates the rate of transport of the compound from the apical side to the basolateral side over time when the rate of transport is linear (usually between T = 60 and T = 120 min), SA is the surface area of the exposure area (cm^2) and $C_{Api,0}$ is the initial donor concentration of the compound (concentration at apical side at T = 0 in dpm/ml or mg/ml).

To study the absorption of compounds, the presence of an unstirred water layer upon the enterocytes should be prevented by continuous moving of the incubation buffers, either by gassing of the media (Ussing system) or placement of the complete system on a rocker platform (InTESTine™ system, Westerhout et al. 2014). Care must also be taken to prevent nonspecific binding of the test compound, evaporation of the media during incubation, cross-contamination of the test compound to other

chambers (especially in case of gassing of the incubation media). The reusable Ussing system therefore requires thorough cleaning and fine adjustment of the gassing rate prior to performing experiments. The InTESTine™ system inserts are generated from disposable glass material, which reduces nonspecific binding of the compound of interest and risk of contamination of the mounted device. Furthermore, the set-up and horizontal mounting enables the InTESTine™ system to be incubated in a carbogenated, humidified incubator at 37 °C on a rocker platform thereby reducing the unstirred water layer, evaporation and possible foaming (Westerhout et al. 2014).

24.3 Monitoring Viability

Previous studies by Haslam et al. (2011), Rozehnal et al. (2012) and Sjöberg et al. (2013) have used the potential difference (>4 mV), short-circuit current (>100 μA/cm^2), trans epithelial electrical resistance (TEER; >20 Ω cm^2) for continuous monitoring of the viability. Low resistance indicates tissue leakage. Due to the set-up of the Ussing chamber system, these measurements can be performed for each experiment. Other markers that can be studied in parallel to a typical transport study include the intestinal metabolism of testosterone and midazolam, by measuring the presence of metabolites apically, basolaterally and intracellularly by means of, for instance, High Pressure Liquid Chromatography (HPLC) (Sjöberg et al. 2013). Transport via the paracellular pathway can be assessed by measuring the permeability of Lucifer yellow (Papp value $<6\times10^{-6}$ cm/s) (Rozehnal et al. 2012) leakage of fluorescein isothiocyanate dextran to the basolateral side (FD4; <0.5 %) (Westerhout et al. 2014), or the linearity of transport of atenolol ($R^2>0.995$ with at least 3 datapoints) (Haslam et al. 2011). Values higher than the ones mentioned above, indicate leakage of the intestinal tissue. Ideally, one would monitor multiple parameters to evaluate the viability and integrity of the intestinal mucosa in parallel to the transport of the compound of interest, but in some cases the compound of interest has the same properties as the marker compounds (e.g. when using fluorescently labeled or radiolabeled compounds).

24.4 Use of Digested Food Samples

While we have shown that undiluted digested food samples (including bile, pancreatic enzymes and/or food components), obtained by using TNO's dynamic computer-controlled in vitro gastrointestinal model of the stomach and small intestine (TIM-1, Minekus et al. 1995) can be applied directly onto intestinal tissue segments (Westerhout et al. 2014), it must be noted that the digested food samples can also be presented in a different formulation. Care should be taken that the levels of alcohol and DMSO are below 1 % and other conditions, such as bile salt concentration, osmolarity and pH, are in concordance to what has been presented by Bergström

et al. (2014). In general, bile salt concentrations should be between 1.4 and 24 mM, the osmolarity should be between 137 and 416 mOsm and the pH should be between 5.4 and 7.8.

24.5 Readout of the Ussing Chamber System

Since the Ussing chamber system is not often applied for the investigation of the impact of food bioactives (proteins, sugars, lipids) on health it is difficult to identify typical readout parameters. To date, the Ussing chamber has been applied to investigate the general transport of proteins, sugars and lipids across intestinal tissue (Table 24.1). The readout for these types of studies is the percentage of the applied dose that can be detected in the basolateral compartment intact using for instance radiolabel counting (e.g. ^{3}H, ^{125}I, ^{14}C), HPLC coupled to a mass spectrometer or UV

Table 24.1 Different absorption studies using intestinal tissue segments of different species mounted in the Ussing chamber system

References	Species	Protein	Sugar	Lipid	Food-related	Analysis
Ducroc et al. (1983)	Rabbit	HRP			No	Enzymatic activity, ^{3}H-radioactive labeling
Gabler (2009)	Pig		Glucose		Yes	Potential difference
Hardin et al. (1999)	Rabbit	BSA			No	Immunoblotting, ELISA and ^{125}I-radioactive labeling
Heyman et al. (1984)	Human (0.5–13 years)	HRP			No	Enzymatic activity and ^{3}H-radioactive labeling
Herrmann et al. (2012)	Pig		Glucose		Yes	Potential difference
Keljo and Hamilton (1983)	Pig	HRP			No	Enzymatic activity
Larsen et al. (2001)	Human		Glucose		Yes	Potential difference
Majamaa and Isolauri (1996)	Human (0.5–8 years)	HRP			No	Enzymatic activity and ^{125}I-radioactive labeling
Schulthess et al. (1996)	Rabbit			Cholesterol	Yes	Merckotest
Sjöberg et al. (2013)	Human (adults)		Glucose		Yes	^{14}C-radioactive labeling
Sutas et al. (1997)	Wistar rat pups	HRP			No	^{125}I-radioactive labeling and HPLC gel filtration

Table 24.2 General advantages, disadvantages and limitations of the Ussing chamber system

Advantages	• Use of ex vivo (human) intestinal tissue, which contain the morphological and physiological features of the intestine • Possibility to study regional (duodenum, jejunum, ileum or colon) absorption and immune responses • Animal tissue allows the possibility to investigate the effect of sensitization on the intestinal absorption of proteins by immunizing the tissue in vivo prior to the in vitro studies (Walker et al. 1974)
Disadvantages	• Viability of the tissue segments up to 150 min • Limited availability of healthy human tissue. Alternatively, animal tissue can be used, but the extrapolation from animals to humans remains to be investigated • Large inter-individual variability, making it difficult to interpret results
Limitations	• Relatively low throughput • Labor intensive

detector or enzymatic activity. While horseradish peroxidase (HRP) and BSA are not food-related, at least it shows that the Ussing chamber technique can be applied to investigate the transport of proteins across intestinal tissue. At TNO we are currently investigating the possibility to use porcine intestinal tissue segments for studying the transport of different allergens across intestinal tissue. Furthermore, we also use porcine intestinal tissue segments to monitor cytokine release at mRNA levels, since cytokine release at protein level is limited due to viability issue's mentioned before.

24.6 General Advantages, Disadvantages and Limitations of the Ussing Chamber System

The Ussing chamber system is, to date, not often applied for the investigation of the impact of food bioactives (proteins, sugars, lipids) on health. This indicates that still a lot of research can be done in this field. The general advantages and disadvantages of the Ussing chamber system are summarized in Table 24.2.

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