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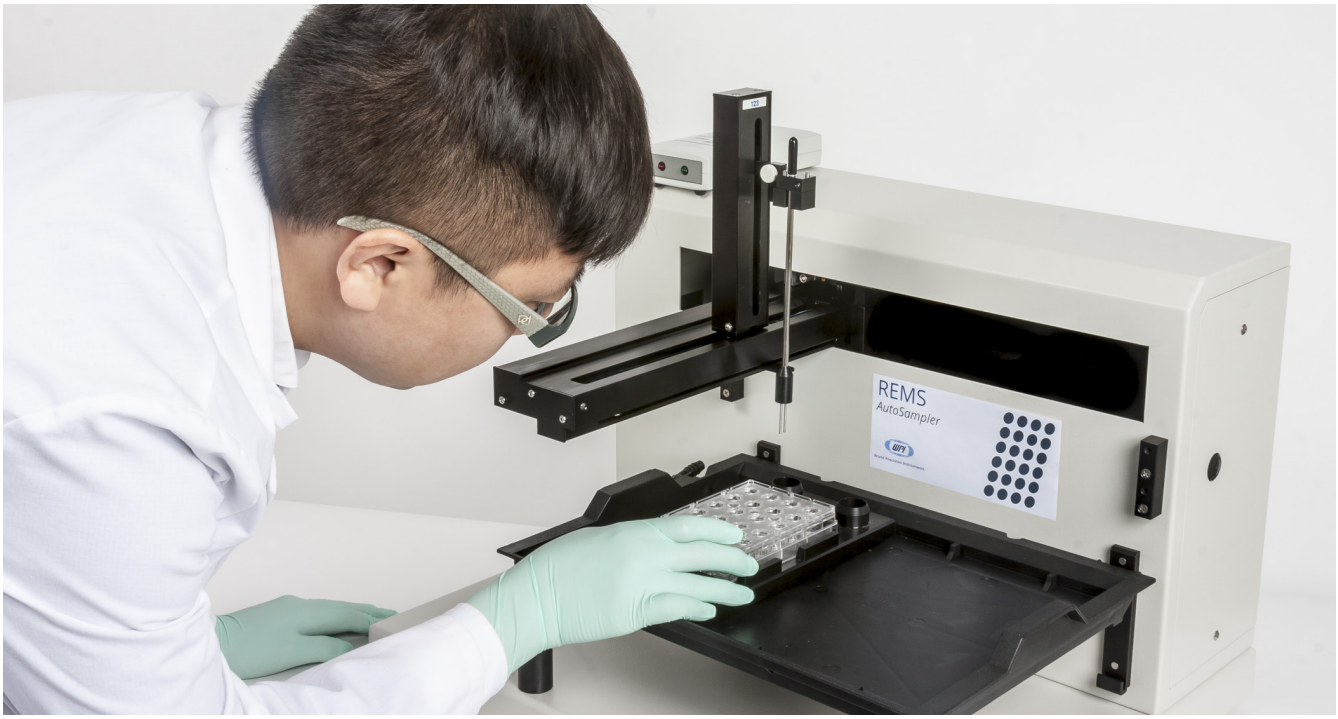
Application Note

REMS AutoSampler

Automated TEER Measurement System



Introduction

The *in vitro* cell culture models of human endothelial and epithelial monolayers are considered as a replica of the *in vivo* environments and as such are used for drug toxicity and transport studies. Findings of the *in vitro* models are also translated to ascertain the metabolic and physiological functions of a particular pharmacological entity. The most prominent endothelial/epithelial *in vitro* models developed in the past are:

- Blood-brain model
- Gastrointestinal tract
- Pulmonary models

These are used for understanding the distribution of drugs, as well as associated toxicities in the organs like the central nervous system, intestine and lungs. These models are either comprised of primary cells or established cell lines. For utilization of these models, the most important feature is to ascertain the capability of cells to form necessary intercellular junctions. Before proceeding with particular drug toxicity or transport the formation of cellular junctions in these *in vitro* models is confirmed through a variety of methodologies including permeability of established barrier to compounds like sucrose having

radiolabeled carbon, and lower molecular weight paracellular tracers including inulin, mannitol and albumin. Specific studies have also reported the uses of nonradioactive fluorescence-labeled marker proteins like dextran labeled with fluorescein isothiocyanate (FITC) and Evans blue dye/biotin-albumin. All these techniques for establishing the integrity of endothelial/epithelial barriers are cumbersome, lacking absolute specificity and the overall impact of these externally added compounds might be influencing the physiological functioning of the barrier established *in vitro* [1].

Besides all the above described methodologies for determining the integrity of an *in vitro* endothelial/epithelial barriers, transepithelial/transendothelial electrical resistance (TEER) measurements are considered more precise and highly quantitative in nature [2]. More importantly the TEER measurements are faster, inflicting no damage to the cells and with nothing being added externally, as the addition of any tracer or radiolabeled compound can potentially change the physiological aspects of cells forming monolayers.

The REMS AutoSampler by WPI is a unique TEER Measurement System that is considered highly reliable for evaluating the integrity of *in vitro* epithelial barrier models including blood-brain barrier, gastrointestinal tract and pulmonary model [3].

TEER Measurement with REMS AutoSampler (Automated TEER Measurement System)

The TEER methods for measuring the integrity of endothelial/epithelial barrier is based on assessing the electrical resistance of the cellular barrier growing in the form of a monolayer. The technique relies on applying the AC electrical signals through electrodes which are inserted on both sides of the cellular monolayer followed by current or voltage measurements.

The REMS AutoSampler offered by WPI measures the electrical resistance of transepithelial/ transendothelial cells grown to confluence on microporous filters of high throughput screening (HTS) 24- and 96-well microplates. The system is automated and as such avoids the user-related issues, generating highly reliable data and enhanced reproducibility. The system operation is PC-controlled, and automated measurement of tissue resistance in cell culture microplates provides the critical advantages of speed, precision, decreased opportunity for contamination and the instant availability of measured resistance data on a computer. These measurements are useful in applications such as evaluating drug toxicity, and bioavailability studies.

The REMS AutoSampler is used for TEER measurements of cells grown in 24- or 96- well HTS microplates. This equipment is capable of making *in vitro* tissue resistance measurements up to 20 kΩ. This system has been used to measure TEER in the following epithelial barrier systems.

i. Blood Brain Barrier

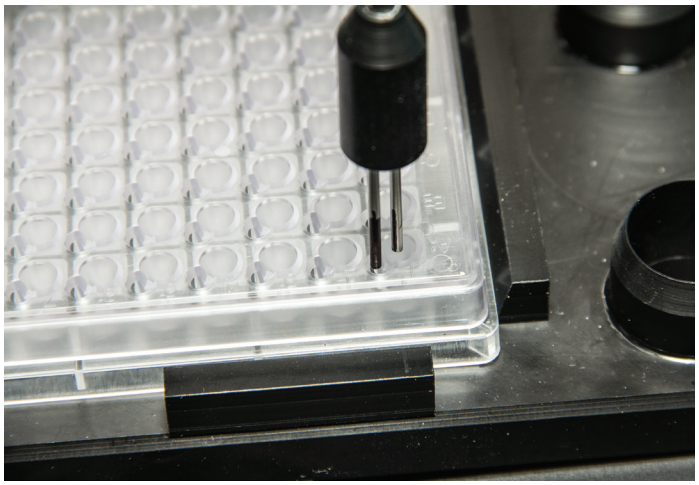
There are several *in vitro* models of the human blood-brain barrier. This biological barrier separates the central nervous system from the systemic circulations [4]. The *in vitro* setup is established by growing brain endothelial cells in conjunction with astrocytes that helps in strengthening the barrier. A variety of pharmacological agents can penetrate into the brain exerting neurotoxicity besides their cytotoxicity on the barrier itself. As such the *in vitro* system is considered as one of the most reliable setups to evaluate the penetration of pharmaceuticals that might exert toxic effects on the central nervous system or even on the barrier properties.

ii. Gastrointestinal Tract (GIT) Model

The epithelial layer of the gastric mucosa comprised of an epithelial layer, lamina propria, and muscularis mucosa limits the drug permeation into the systemic circulation and as such the *in vitro* model of GIT is used for studying the passage of drugs and the penetration of toxic elements. The Caco-2 cell line derived from the human colorectal adenocarcinoma is widely used cell line as an *in vitro* model of the GI tract for evaluating pharmacological passage [5]. Human primary GI tract cells isolated from patients' samples have even been used to establish a GI epithelial barrier model.

iii. Pulmonary Models

The pulmonary barrier models are used for understanding the direct toxicity of pharmaceuticals as well as transport of nutrients and pharmacological molecules into the lungs. The lungs are covered with a continuous epithelium comprised of the airway and alveolar epithelia. Both the airway and alveolar *in vitro* epithelial models have been reported in the literature [6].



Drug Discovery/Toxicology

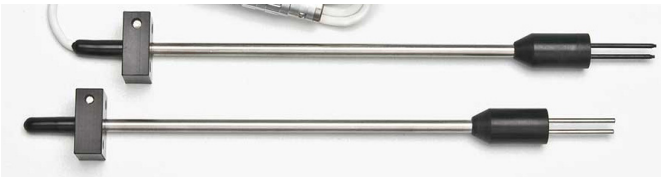
The epithelial membranous structures separate individual compartments of the human body, besides shielding the organs like brain, intestine and lungs. They also play a crucial role in homeostasis. The toxic effect of newly developed/already existing drug modalities on these biological barriers is an active area of research [7]. For evaluating the distribution of pharmacological agents and their relevant toxicity to the *in vitro* barrier models are providing a wealth of information. To assess the barrier properties of cultured cells, it is important that cells establish intercellular junctions like *in vivo* environments. The TEER method is considered fast, accurate and non-invasive, the prime choice of all the available methods. Notably, for evaluating pharmacological transport across these barriers, this method does not add any additional molecule in the system. The REMS AutoSampler has the capability to measure the electrical resistance of transepithelial/transendothelial cellular layers growing to confluence on microporous filters. For high throughput screening (HTS), the instrument has the versatility for use in both 24- and 96-well microplates.

Conclusions

The TEER measurements in the *in vitro* endothelial/epithelial barrier models, i.e., blood-brain barrier models, gastrointestinal tract models and pulmonary models, can be efficiently used to evaluate pharmacological modality toxicities, as well as have applications in drug discovery. The REMS AutoSampler by WPI provides the opportunity for such studies through a non-invasive methodology, which closely mimic the *in vivo* environments.

Automated TEER Measurement System

System Configuration



- The robotic sampler that moves the electrode over each well of the microplate
- An electrode located on the robotic arm
- Base plate for the 24- and 96-well tray
- The windows-based data acquisition card
- REMS interface unit
- REMS software to operate the system on a Windows-based computer
- PC with Windows 7 or higher

References

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