



Epithelial Volt/Ohm Meter

Application: Cellular confluency of epithelial cells

Methods/Overview

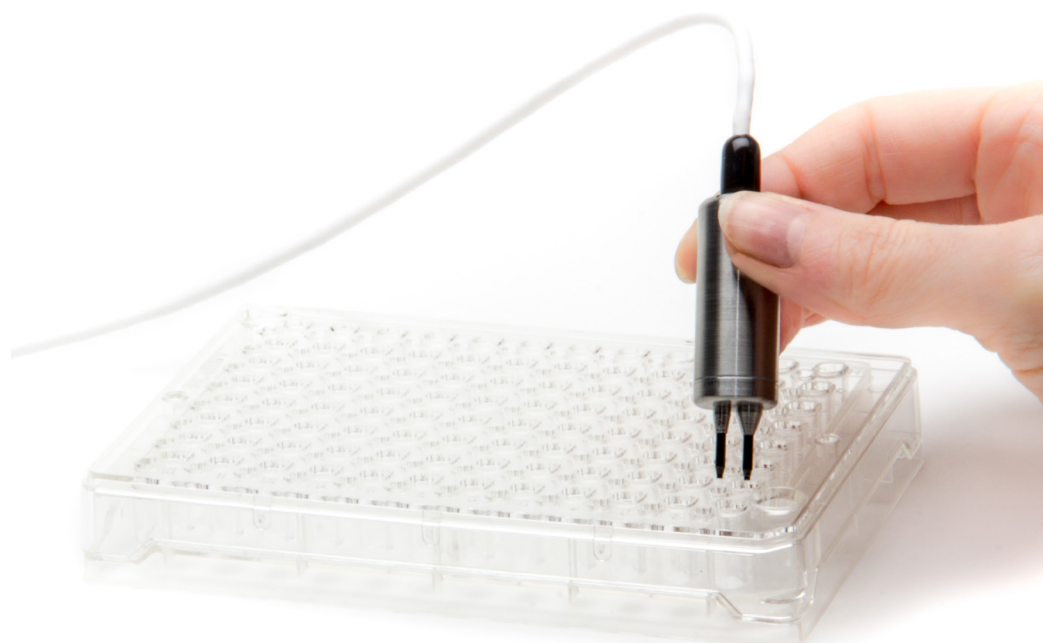
Cell culture is a technique by which individual cells can be isolated from eukaryotic tissues and grown under controlled conditions in the presence of essential nutrients, gases and other physiological requirements. The nutritional requirements for cell culture vary based on the type of cells being cultured in laboratory conditions. Although cells (primary cells) isolated from the tissues have a finite life, a variety of cell lines have been developed with a capability to grow indefinitely exactly like the cancer cells.

There are two main types of cells:

- Suspension cells and
- Adherent cells.

Both these cell types need appropriate care when growing them in the laboratory, however, the adherent cells, also known as epithelial/endothelial cells, need a specific attachment surface for their growth. Confluence is a term used to define the relative area covered by the cells in a particular vessel (flasks, cell culture dish, etc.). A 50% confluency means that half the area of the container or dish has cells, and the other half is still available for the cells to grow. 100% confluence means no space is left over in between the cells within the vessel being used for cell culture.

For adherent cells, the confluence in the *in vitro* conditions has important implications in several studies involving drug transport, tissue engineering and cell stock maintenance. Particularly, clinical application of cells necessitates appropriate, non-invasive methodologies



for defining cell confluence¹. Several protocols have been established for the measurements of cell confluence. Among these are a few worth describing:

1. **Live Cells Tissue Culture Microscopy** – In almost every cell culture laboratory, cell confluence is defined through subjective methodologies. The live cell microscope (having its objective lenses at the bottom of cell culture vessels) provides an opportunity to focus on the cell being grown in the tissue culture vessels. Cell confluence is defined by visual observation, defining the result through approximation. Although a sophisticated confluence measurement with quantitative methodology takes into consideration the area populated by the cells over the total area of the field of view².



2. **Confluence Measurements through Phase Contrast Microscopy** – In phase contrast microscopy, the relative confluence of cells is based on the cellular translucence. The cellular boundaries are the measure of the confluency³. This technique has been further refined, and the confluence of cells is determined indirectly by the area fraction (AF) output through digital photography methodologies. The method involves capturing the image with a standard inverted phase contrast microscope equipped with a digital camera followed by its analysis through ImageJ software⁴.
3. **Holographic Microscopy for Confluence Measurement** – This method requires a specialized microscope and imaging system, holomonitor (Phase Holographic Imaging AB, Lund, Sweden). This setup combines digital holography with phase contrast microscopy for gathering information about the confluence of adherent cells⁵.
4. **Fluorescein isothiocyanate (FITC) labeled bovine serum albumin (BSA)/Dextran passage through a monolayer of cells⁶** – This methodology is only used for ascertaining the barrier properties of the epithelial/endothelial cell monolayer. When the cells cover an entire surface area of the cell culture vessels, only then FITC labeled biomolecules can be used.
5. **Trans-epithelial/trans-endothelial electrical resistance (TEER)** – The TEER measurement methodology is the most widely used method for ascertaining the 100% confluence of cells associated with their functional aspects. A simple instrument (EVOM) was initially designed to provide information about the confluence of cells⁷. This has been refined with a second generation (EVOM2). It is used in conjunction with a highly sensitive electrode (STX2). The electrode has two parts, one inserted in the apical surface and the other on the basal side of the chamber having epithelial/endothelial cells. When the cells growing in the chamber attain 100% confluence, they manifest different levels of resistance when compared with lower confluence. The EVOM2 meter is an advanced version of the first generation equipment, and it can be calibrated easily for measuring the transepithelial electrical resistance (TEER). The calibration process involves utilizing standard 1000 Ω test resistor provided with the equipment.

Pros and cons of each method

In describing the advantages and disadvantages of methods used for the measurements of cellular confluence, each and every method has its advantage. Basically, it is the experimental setup that defines which type of methodology should be utilized and whether confluence matters in the experimental setup being used. For example, the live cell microscopy and phase-contrast (individually or aided with image analysis software) are suitable if the cells are being grown in culture vessels, and the ultimate aim is not to study their physiological barrier properties. The same is the case with the holographic methodologies. The quantitative phase microscopy to determine confluence is a quick procedure utilizing a non-destructive approach, however, it has a limitation in providing barrier properties of the cells being cultured³. Utilization of holography needs sophisticated software and equipment (Holomonitor™) for computing amplitude and images captured through the hologram. And, it requires highly trained technical manpower for deciphering the information. However, it offers information about the cell number and their size through non-invasive methodologies.

For experimental setups in which the barrier properties of the cell being grown are evaluated, there is nothing better than the TEER measurements using World Precision Instrument's EVOM2. Although, the researcher needs to calibrate the equipment for cell type and the chamber/vessel being utilized for cell growth. The resistance values manifested at 100% confluence are expressed in ohms, and they vary based on the type of cells examined. The reason for these variations is that endothelial cells forming various body organs differ in their barrier properties. A cell growth optimization should be followed by their evaluations. Although fluorescently labeled albumin, dextran and several other biomolecules can be used for ascertaining the confluence of cells, these methodologies do not provide conclusive information about the barrier properties of the cells being evaluated.

The value of using TEER measurement

The cell is the basic unit of life, and a group of cells unites together to form the tissues, which combine to form organs that are comprised of different types of cells. In defining the value of TEER measurement relevant to cell confluence, one has to understand that tissues are performing several functions, and any deformity or any diseased, dead or sick (necrotic) cells compromise the integrity of the tissue. Compromised tissues lose proper physiological functioning.

The TEER measurement basically defines and characterizes a functional tissue *in vitro*. Although several methodologies exist for determining the cell confluence, TEER measurement is the only methodology that has physiological relevance. For example, in blood-brain-barrier (BBB) modeling for understanding the passage of infectious agents or pharmaceutical agents transport through these barriers, the preliminary requirement is the establishment of an intact *in vitro* BBB having 100% cellular confluence in the defined area with its functionality mimicking *in vivo*. Under such circumstances, live cells, phase contrast or holographic microscopy will definitely give information that cells are 100% confluent. However, it is impossible to have meaningful data as far as the physiological function is concerned⁸.

Several studies have used TEER methodologies to ascertain 100% cellular confluence associated with barrier properties for understanding drug transport into the central nervous system (CNS) particularly for brain tumors⁹. However, epithelial cell/endothelial cell barriers are not only in the BBB, but rather several other tissues also have such system for controlling the movement of various biological agents in and out of the organs. The *in vitro* TEER measurements helped in defining the etiological factors involved in acute chest infection manifested through disruption of lung microvascular endothelial cells compromising this important barrier, i.e. cellular confluence¹⁰. Already, there is an existing vast list of scientific studies utilizing TEER measurement methodologies in defining the pathological aspects of several diseases including diabetic retinopathy and weakened endothelial blood-retinal barrier¹¹, sepsis studies associated with deformity of the endothelial barriers in various organs of the body¹² and the list goes on. Furthermore, the tissue engineering for clinical



purposes has opened up a new era focusing on cellular confluence, as well as their physiological properties, where TEER is supposed to play a significant role.

Reasons to trust the EVOM2 (from WPI) in cellular confluence measurements

Several reasons and available data indicate the use of the EVOM2 from World Precision Instrument for determining cellular confluence. First, and foremost, the TEER measurements are relatively safe and avoid contamination of the cells being studied. Second, there is no stress on the living cells during the usage of the EVOM2, and sometimes highly precious primary cells that are hard to procure can be saved. Third, the data generated by using EVOM2 is more realistic and mimics the physiological patterns *in vivo*. The findings of studies in which barrier properties (100% confluence) are being determined are further confirmed in the animal model. The EVOM2, besides measuring the cellular confluence of cell monolayers, also determines the health manifested through the barrier properties that none of the other existing methodologies could. As of now the only equipment that provides an opportunity for scientists to determine 100% cellular confluence of epithelial cells/endothelial cells associated with physiological functioning is the EVOM2 by the World Precision Instruments. A careful look at the studies evaluating barrier properties *in vitro* for human tissues linked with cellular confluence in the past few years have used TEER measurement, the most authentic measure when compared with other methodologies.

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References

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