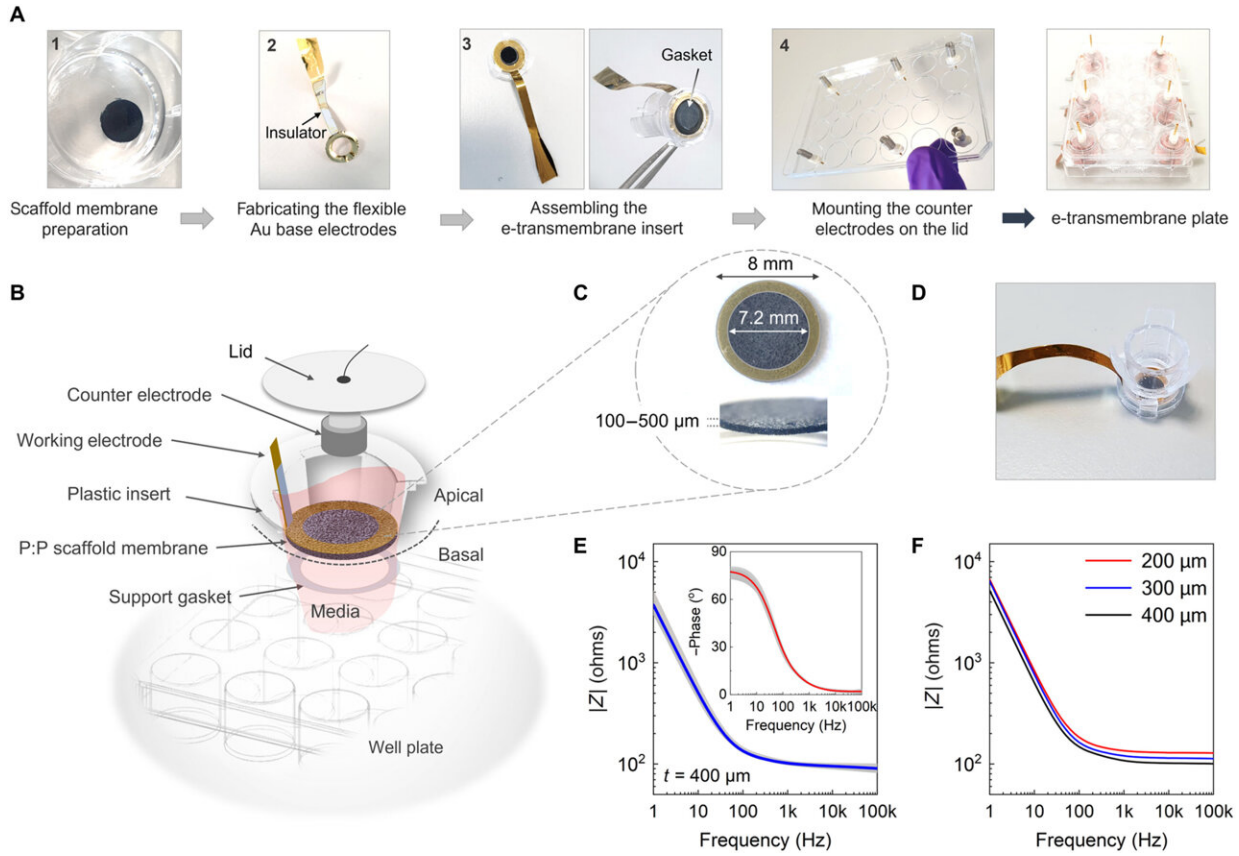
# Developing an organic transmembrane device to host and monitor 3-D cell cultures

October 12 2022, by Thamarasee Jeewandara




The concept of the e-transmembrane platform. (A) Photographs showing the processing steps for the fabrication of the e-transmembrane platform. (B) Schematic illustration of the bioelectronic insert showing the various critical components of the device. The sectional structure clearly shows the basal and the apical domains and the geometrical features of the electrodes. The working electrode (WE) module is composed of a gold (Au) base electrode attached to the PEDOT:PSS scaffold membrane. The counter electrode (CE) is a high

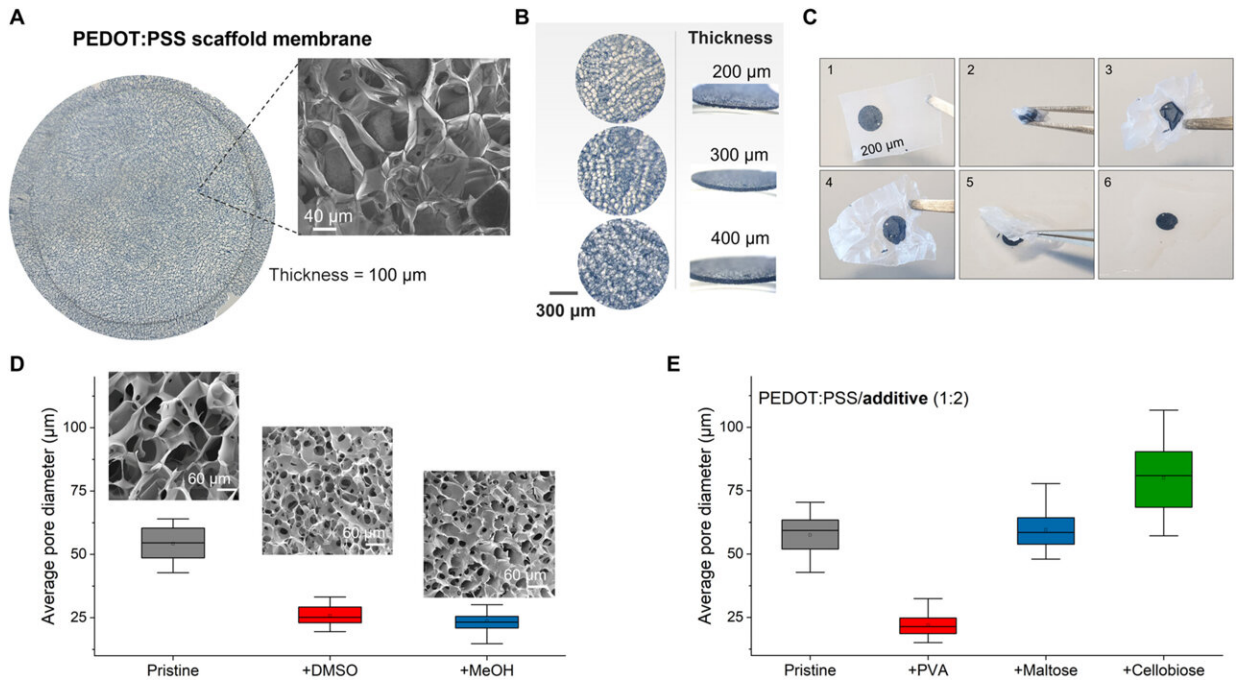
surface area metal woven mesh attached to the lid of the well plate. The insert setup shown here is compatible with a 24-well plate configuration. (C) Optical micrograph of a PEDOT:PSS e-transmembrane. The thickness of the membrane typically ranges between 100 and 500 μm, and its diameter is 8 mm. The aperture is defined by the inner diameter of the Au O-ring electrode and, in the present configuration, is approximately 7.2 mm. (D) Photograph of the fully assembled device. (E) Electrochemical impedance spectroscopy measurements showing the Bode magnitude (|Z| – f) and the corresponding phase plots (insert) measured from multiple devices (of 400 μm thickness, two batches, N = 5). The solid blue and red lines represent the generated averaging curves. (F) Bode plot spectra of e-transmembrane electrodes of various thicknesses (200, 300, and 400 μm). Note that EIS measurements were carried out in cell culture medium. Credit: *Science Advances* (2022). DOI: 10.1126/sciadv.abo4761

Researchers have used 3D cell culture models in the past decade to translate molecular targets during drug discovery processes to thereby transition from an existing predominantly 2D culture environment. In a new report now published in *Science Advances*, Charalampos Pitsalidis and a research team in physics and chemical engineering at the University of Science and Technology in Abu Dhabi, UAE and the University of Cambridge describe a multi-well plate bioelectronic platform named the e-transmembrane to support and monitor complex 3D cell architectures.

The team microengineered the scaffolds using poly(3,4-ethylenedioxythiophene polystyrene sulfonate to function as separating membranes to isolate cell cultures and achieve real-time in situ recordings of cell growth and function. The high surface area to volume ratio allowed them to generate deep stratified tissues in a porous architecture. The platform is applicable as a universal resource for biologists to conduct next-generation high-throughput drug screening assays.

# A new e-transmembrane platform for drug screening and drug discovery

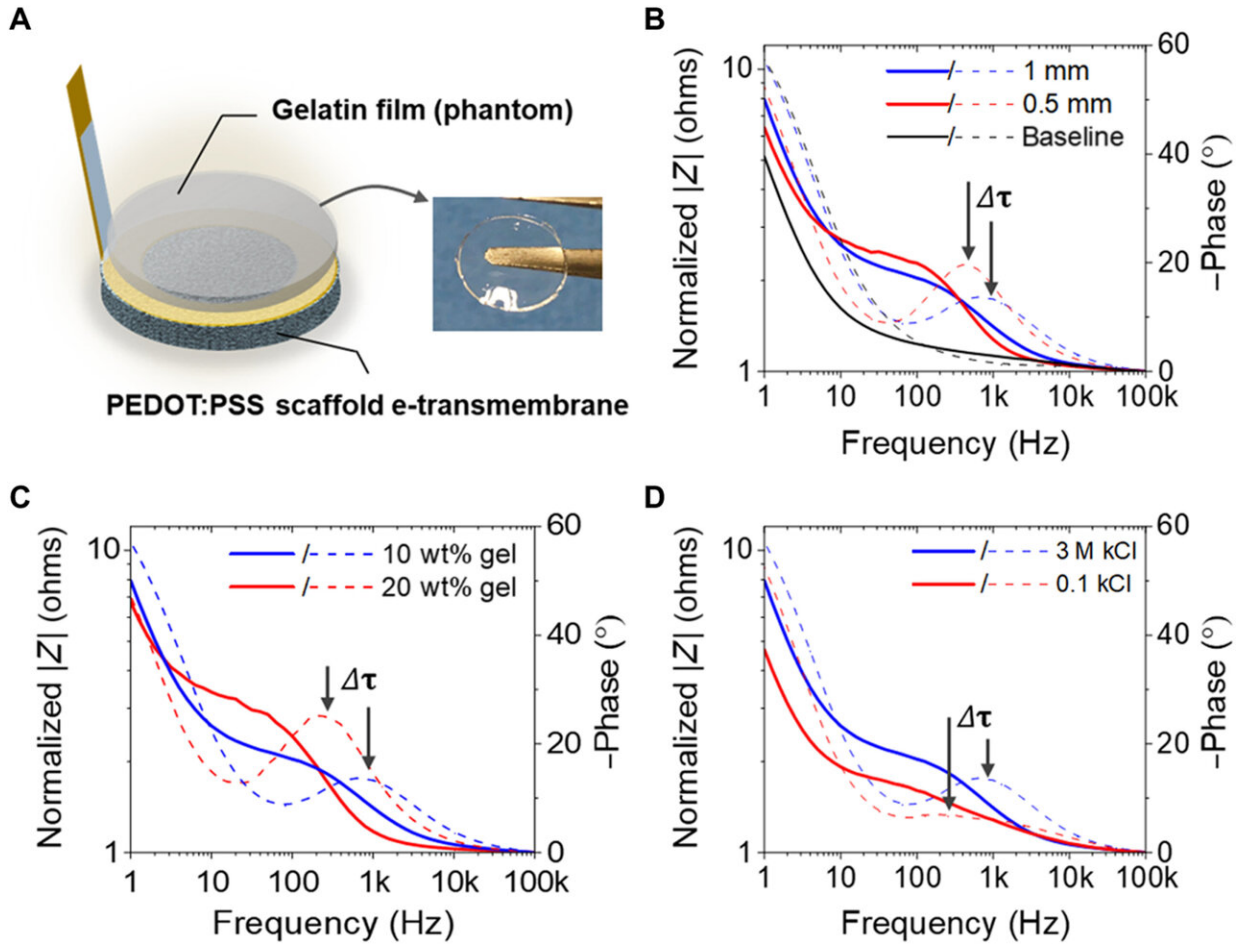
The existing knowledge of cell growth, function and homeostasis arises from two-dimensional cell-based assays using cell monolayers grown on flat, rigid substrates. While such assays are applicable across fundamental research and toxicology screening, they do not adequately demonstrate the complex 3D microenvironment observed in vivo, as noted in cell-cell and cell-extracellular matrix interactions. While animal studies are regarded as a gold standard for preclinical studies, they are limited by marked physiological differences between species, costs and ethical concerns. Researchers have therefore shifted the focus to improve in vitro systems such as 3D cell cultures and organ-on-a-chip devices to better emulate physiological architectures of biological systems in vivo.




Morphological properties of the PEDOT:PSS e-transmembranes. (A) Optical

micrograph of a 100-μm-thick scaffold membrane and SEM image of the corresponding pore morphology. (B) Optical micrographs showing top and side views of PEDOT:PSS e-transmembranes of various thicknesses (200, 300, and 400 μm). (C) Sequential photos showing the macroscopic effects of crumpling-unfolding test on PEDOT:PSS e-transmembranes. To perform the test, a 200-μm-thick membrane is attached to a parafilm tape and then subjected to a random macroscopic deformation. (D) Tailoring the pore size of the PEDOT:PSS e-transmembranes using inhibitors of the freezing process. Pristine PEDOT:PSS solution is mixed with dimethyl sulfoxide (DMSO) or methanol (MeOH) at 1 and 2.0% (v/v) concentration, respectively. The graph shows the corresponding mean pore diameter approximated from at least N = 20 pores using image analysis in the SEM images. (E) e-transmembrane composites based on mixtures of PEDOT:PSS with polyvinyl alcohol (PVA) or with oligosaccharides (maltose and cellobiose). The ratio of PEDOT:PSS/additive is 1:2. The graph shows the mean pore diameter approximated from at least N = 20 pores. Credit: *Science Advances* (2022). DOI: 10.1126/sciadv.abo4761

In recent studies, Pitsalidis and the team used conducting polymer scaffolds as architectures to host 3D cell cultures for real-time sensing. In this work, they developed a bioelectronic transmembrane device to combine a range of desirable features, including the potential to host biologically complex and physiologically relevant 3D cell co-cultures and monitor the models in real-time. The researchers propose using the "e-transmembrane" platform as a highly useful resource for drug discovery.



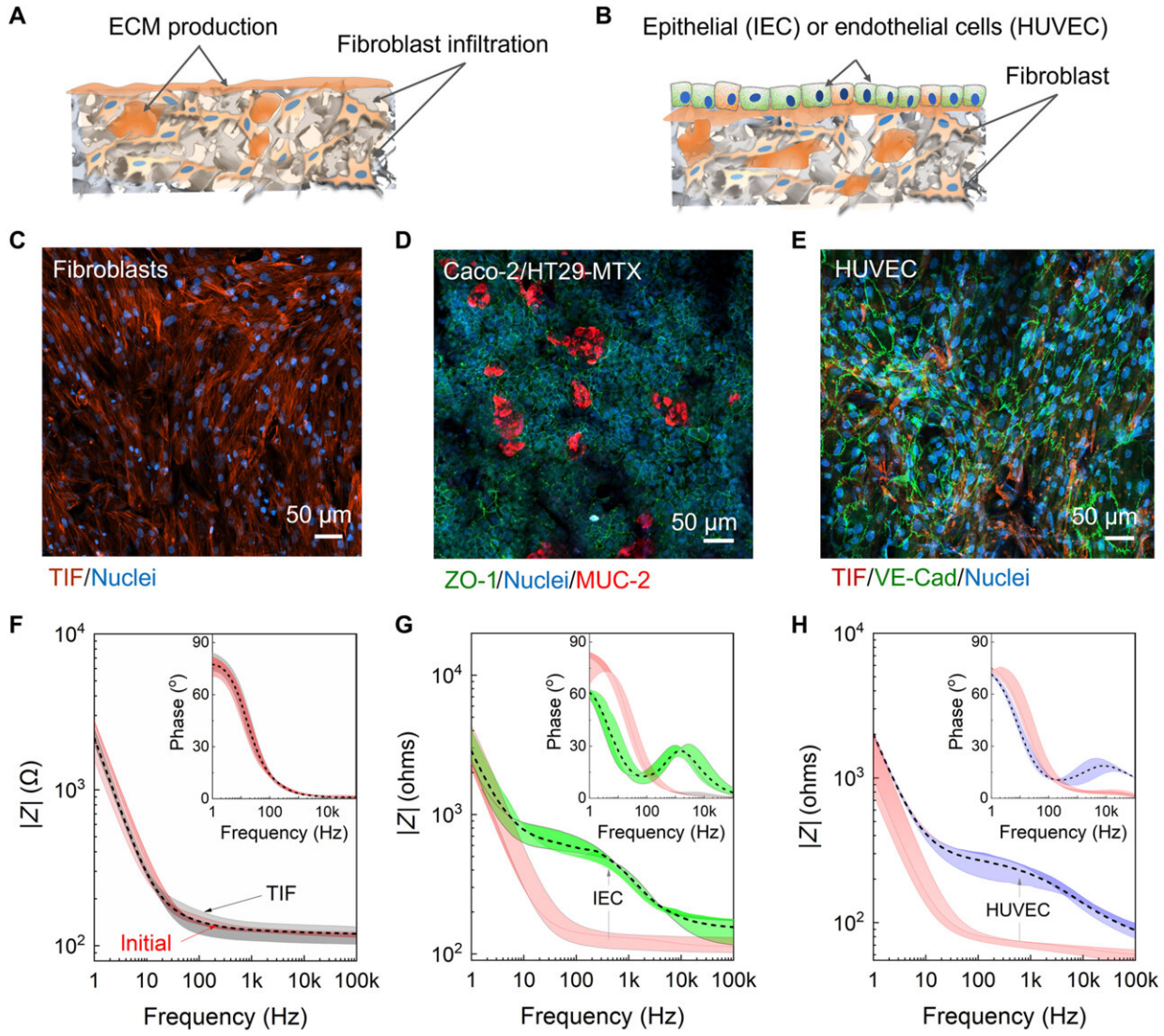

Validating the electrochemical operation of the e-transmembrane device using gelatin phantom inserts. (A) Schematic representation of the e-transmembrane with the phantom installed (i.e., a gelatin film clamped onto the apical side of the PEDOT:PSS scaffold). Varying physical and chemical properties of the phantom changes its resistivity or permittivity (capacitance), which will cause a shift in the time constant ($\tau = RC$) of the parallel R-C impedance of the phantom. Comparative impedance Bode plots of the gelatin phantoms (B) for various thicknesses (0.5 mm versus 1 mm), showing a time constant to shift to lower frequencies that indicates an increase in the capacitance with decreased thickness. (C) Increasing the gelatin content [from 10 to 20 weight % (wt%)] of the phantom electrode increases the permittivity, which increases the capacitance and causes the time constant to shift to a lower frequency. (D) Decreasing the KCl concentration from 3 to 0.1 M increases the resistivity, which causes the time constant to shift to a lower frequency. [Solid lines correspond to impedance magnitude (|Z|), and dashed lines correspond to impedance phase; black data

series correspond to the blank device without the gelatin phantom.] Credit: *Science Advances* (2022). DOI: 10.1126/sciadv.abo4761

## Developing the e-transmembrane platform in the lab

The scientists developed the e-transmembrane by engineering three key modules; the conducting polymer scaffold membrane, electrical interconnects to capture electrochemical readouts and plastic insert components. They developed an e-transmembrane system as a two-dimensional electrochemical device to conduct electrochemical impedance spectroscopy (EIS) measurements. Due to the presence of the physiological media of the cell culture, the electrochemical behavior underwent alterations; however, these variations were negligible after two weeks in cell culture media.

The team next explored the use of scaffold membranes in an organic electrochemical transistor in order to investigate the transistor mode of functionality via performance optimization studies in the future. They conducted a series of experiments to show that the mechanical properties of the 3D constructs influenced cell-substrate interactions. Thereafter, by tailoring the pore size and morphology, the researchers regulated the functionality of the constructs.



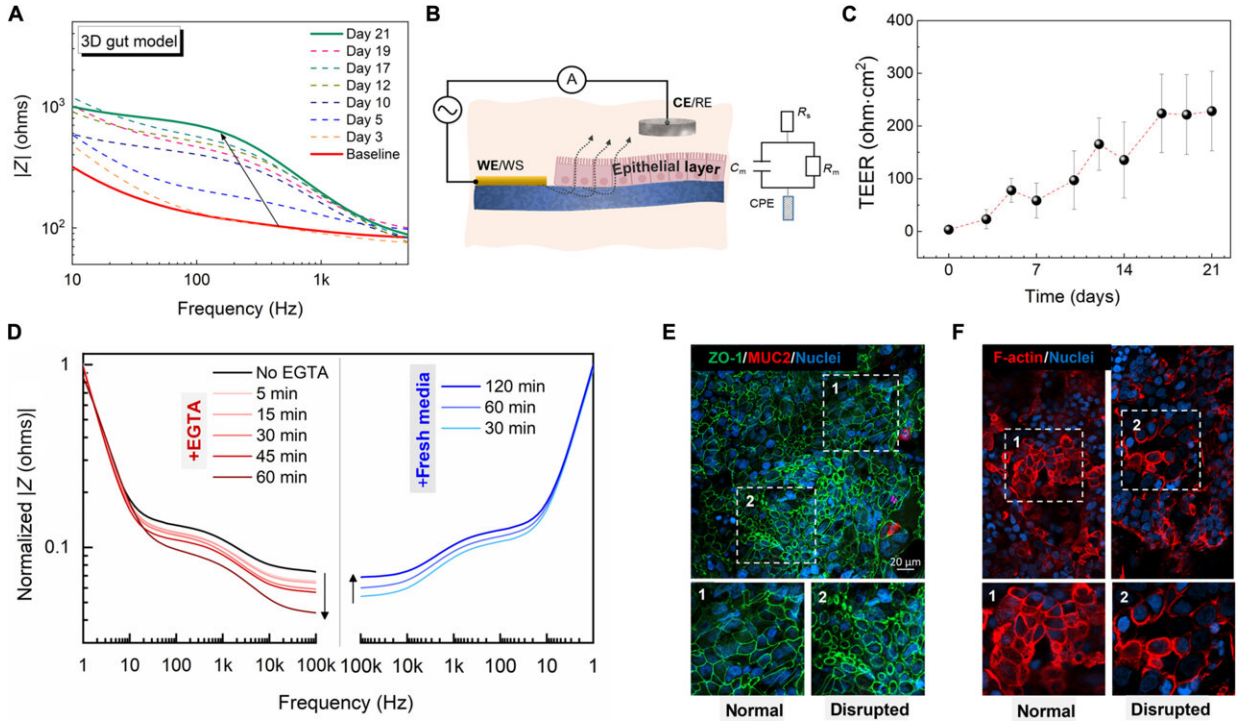

3D cell culture experiments and EIS monitoring using e-transmembrane platform. (A) Illustration of tissue formation within the e-transmembrane to support (B) 3D epithelial or endothelial models. (C) Confocal images of fibroblasts (TIFs; actin cytoskeleton in red), counterstained for nuclei (blue), demonstrating cell infiltration and fibrillar network (21 days of TIF monoculture). (D) Immunofluorescence of intestinal epithelium, illustrating ZO-1 protein (green), revealing the formation of the tight junction network and secretion of MUC2 (red), and nuclei (blue; see also fig. S4). (E) Immunofluorescence image of human umbilical vein endothelial cells (HUVECs; 14 days) over a fibroblast-cultivated e-transmembrane. Images shows adherens junctions [vascular endothelial (VE)–cadherin in green]. Cells were

counterstained for nuclei (blue) visible over a layer of fibroblasts (actin in red; a 500-μm e-transmembrane was used for the HUVEC experiments). Bode plots of the models showing the impedance magnitude from (N = 3) different devices and/or experiments. The color bands represent superimposed EIS plots for various devices. The dashed line corresponds to the average curving. (F) Fibroblasts (gray band, TIF, 5 days) – initial (uncultured e-transmembranes) and (G) IECs (green band, Caco-2/HT29-MTX, 21 days) overlaying fibroblast-cultured e-transmembrane (red band, TIF, 5 days). (H) Endothelial cells (blue band, HUVEC, 14 days) overlaying a fibroblast-cultured e-transmembrane (red band, TIF, 5 days). Insets show corresponding phase plot for models for the different devices. Credit: *Science Advances* (2022). DOI: 10.1126/sciadv.abo4761

## From a 3D cell culture system to a 3D human intestine on an e-transmembrane

The team then modeled the e-transmembrane electrode functionality to assess cell barrier integrity, and qualitatively observed the morphology of the measured impedance and relative changes by using 3D cell culture systems. The typical e-transmembrane model contained human fibroblasts grown in the bulk of the scaffold with a confluent monolayer of human epithelial and endothelial cells seeded on the top. The scientists cultured the electroactive substances with human fibroblasts to serve as a guide for tissue organization with subsequent integration of cell types that varied according to their tissue model of interest.

The researchers used these study outcomes to develop a 3D human intestine on an e-transmembrane device. The fibroblasts and their resultant proteins affected cell attachment, polarization and functional properties of epithelia in the triculture model. The team subsequently monitored the real-time cell tissue and barrier integrity to detect and circumvent any breaches in the intestinal barrier.




e-transmembrane platform as a tool for culturing and monitoring a 3D model of the human intestine. (A) Time-resolved impedance spectra of a 3D gut epithelial model developed in the e-transmembrane device. (B) Schematic showing the electrochemical setup of the e-transmembrane and the corresponding equivalent circuit with three elements in series accounting for the electrolyte (Rs), scaffold-membrane (CPE), and the cell layer (Cm//Rm). (C) TEER evolution of the epithelial (Caco-2/HT29-MTX) cell monolayer. Data for the Caco-2/HT29-MTX resistance were generated 4 Science Advances (2022). DOI: 10.1126/sciadv.abo4761

## Outlook

In this way, Charalampos Pitsalidis and colleagues developed a first in-study example of a bioelectric well plate platform by using an e-transmembrane template. The porous platform facilitated 3D cell

cultures and enabled them to monitor cell growth in multiculture systems. The e-transmembrane functioned both as an electrode and a transistor with the capacity to regulate the pore size and morphology of the device for specific applications. The 3D cell culture platform is capable of incubating multicultures of cells for high-throughput measurements as a drug screening and therapeutics platform. The scientists envision implementing the concept to expand interfaces of the tissue to lung and blood brain barrier for drug discovery and disease modeling applications.

**More information:** Charalampos Pitsalidis et al, Organic electronic transmembrane device for hosting and monitoring 3D cell cultures, *Science Advances* (2022). DOI: 10.1126/sciadv.abo4761

Marinke W. van der Helm et al, Non-invasive sensing of transepithelial barrier function and tissue differentiation in organs-on-chips using impedance spectroscopy, *Lab on a Chip* (2019). DOI: 10.1039/C8LC00129D