Transistor in a tube: A route to three-dimensional bioelectronics

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Advances in three-dimensional (3D) cell culture materials and techniques, which more accurately mimic in vivo systems to study biological phenomena, have fostered the development of organ and tissue models. While sophisticated 3D tissues can be generated, technology that can accurately assess the functionality of these complex models in a high-throughput and dynamic manner is not well adapted. Here, we present an organic bioelectronic device based on a conducting polymer scaffold integrated into an electrochemical transistor configuration. This platform supports the dual purpose of enabling 3D cell culture growth and real-time monitoring of the adhesion and growth of cells. We have adapted our system to a 3D tubular geometry facilitating free flow of nutrients, given its relevance in a variety of biological tissues (e.g., vascular, gastrointestinal, and kidney) and processes (e.g., blood flow). This biomimetic transistor in a tube does not require photolithography methods for preparation, allowing facile adaptation to the purpose. We demonstrate that epithelial and fibroblast cells grow readily and form tissue-like architectures within the conducting polymer scaffold that constitutes the channel of the transistor. The process of tissue formation inside the conducting polymer channel gradually modulates the transistor characteristics. Correlating the real-time changes in the steady-state characteristics of the transistor with the growth of the cultured tissue, we extract valuable insights regarding the transients of tissue formation. Our biomimetic platform enabling label-free, dynamic, and in situ measurements illustrates the potential for real-time monitoring of 3D cell culture and compatibility for use in long-term organ-on-chip platforms.

INTRODUCTION

Cell-based assays have been extensively used for drug discovery as well for understanding molecular mechanisms of disease for several decades. Although the majority of techniques rely on optical transducers, electrical transduction is arguably a hugely data-rich and dynamic means of interfacing with cells. Most electrical measurements have so far focused on electrophysiological interfacing with electrogenic cells (e.g., neurons or cardiac tissues) (1, 2). However, a substantial body of work exists using electrical impedance methods to monitor properties of cells as diverse as adhesion to micromotion in a noninvasive, label-free manner, owing to the pioneering work of Giaever and Keese (3). Transistors, thought by many to be a revolutionary invention that enabled the era of microelectronics, can be used as transducers of biological signals when integrated with electrolytes (4). An electrolyte-gated transistor, organic electrochemical transistors (OECTs) have been particularly favored for biotransduction as they can transduce biological signals into electrical output using very low operation voltages (5). The OECT uses an organic semiconductor film in the channel in contact with an electrolyte (biological medium) whose potential is modulated by a gate electrode. The operation of an OECT relies on the penetration of ions of the electrolyte into the channel and their ability to change the doping state, and therefore the conductivity, of the channel. The identifying characteristic of these devices is that the interaction of ions with the channel spans the bulk of the organic electronic channel, which leads to a large amplification of the gate modulation (6).

OECTs have therefore been integrated in a variety of biochemical sensing platforms, including implantable arrays, which record signals from electrically active cells with record-high sensitivities (7), or in vitro platforms, which measure metabolite concentrations in physiological fluids (8–10). Hence, OECTs directly interfacing with cell cultures were shown to assess the integrity and health of barrier-forming (non-electrogenic) cells with a degree of superiority over the traditional electrochemical impedance sensing platforms using electrodes (11). In these measurements, cells growing on the channel impede the ionic flux between the electrolyte and the channel and alter the performance of the device. While the first reported OECTs were based on polypyrrole (12), the workhorse material used typically as the channel is the conducting polymer poly(3,4-ethylenedioxythiophene) doped with poly(styrene sulfonate) (PEDOT:PSS) due to its notable stability in its oxidized and reduced forms. Especially important for cell-based applications, its optical transparency and its amenability to surface functionalization allow parallel optical and electrical assessment of cells (13) as well as controlled cell attachment and growth on its surface (14).

While most biological transducers incorporate [two-dimensional (2D)] monolayers of cells cultured on planar substrates, there is a growing recognition that data gleaned from flat biology approaches can be misleading. 3D approaches to cell culture are now widespread and are being embraced by the organ-on-chip community for human-relevant drug discovery and toxicology assays (15–17). Hence, a large variety of 3D models that better mimic in vivo physiology has been developed, including spheroids, organoids, and scaffolds, with the latter typically being a porous cell support matrix composed of synthetic materials or biopolymers (18, 19). Integration of these complex systems with electrical transducers has been limited to flat electrodes (20–23), not compatible with intimate monitoring of the function of these complex models in a precise manner. However, as recently demonstrated, the ability of conducting polymers to be fabricated into mechanically soft hydrogels and
scaffolds opens exciting new possibilities to integrate electrically conducting materials with 3D cell cultures. Previously, the integration of scaffolds into OECTs was shown, but the large size of the devices meant very slow speeds and low transconductance, and no cell monitoring was shown. We recently showed for the first time the use of PEDOT:PSS scaffolds to simultaneously host and monitor (via impedance) cocultures of mammalian cell growth within its porous architecture. We were inspired by tissue engineering approaches to grow cells in 3D on porous scaffolds with mechanical and biochemical cues enabling cell growth, but with the addition of electrical functionality owing to the conducting polymer. Such a 3D electrode device offers enhanced sensing capabilities; however, extracting the critical parameters from the complex impedance spectrum is rather challenging.

Continuing from this work, here, we show the use of 3D macroporous scaffolds in an OECT configuration for dynamic monitoring of cell culture growth. We demonstrate easy tuning of these scaffolds in terms of electrical, mechanical, and biochemical properties due to an in situ lyophilization process, which we have adapted to assemble scaffolds inside fluidic tubes. The tubular structure facilitates gas exchange and fresh medium delivery to the cells that are grown inside the scaffold, resembling some degree blood vessels. We demonstrate that cells grow readily and form tissue-like architectures within the conducting polymer scaffold constituting the channel of the transistor. The tissue formation gradually modulates the electronic properties of the conducting polymer scaffold, as evidenced by changes in both the steady-state and transient characteristics of the device. Correlating the changes in the performance of the transistor to the adhesion and growth of cells over time, we extract useful information regarding the tissue formation. This label-free, in situ, dynamic, and "living" electronic tool illustrates the potential of these scaffolds for real-time monitoring of 3D cell culture and compatibility with use in long-term organ-on-chip platforms.

RESULTS

The most common and relatively facile technique to shape a polymer into a 3D structure is through freeze drying (known as lyophilization) (29, 30). Using these processes, free-standing macroporous scaffolds can be prepared from aqueous solutions/ dispersion of polymers with mechanical stability and porosity adjusted by the material composition and the processing conditions. Moreover, as the base material is in liquid form, it is feasible to regulate the dimensions of the resulting solidified structure by the mold in which it is cast. This offers unprecedented ease in fabrication as well as great versatility for designing platforms based on these 3D materials with macroscopic pores. In our previous work, a fluidic tubing was integrated with a relatively large PEDOT:PSS-based scaffold (using a cuvette as a mold) to promote homogeneous cell accumulation inside the scaffold as well as provide continuous perfusion of nutrients required for cell growth over several days (31, 32). We have now inverted and miniaturized this concept and designed a 3D cell monitoring platform based on a tubular T-shaped arrangement with the source, drain, and gate electrodes embedded inside the fluidics. This geometry allows the integration of a 3D conducting channel (the 3D conducting polymer scaffold) within the tube, which simultaneously provides perfusion capability, with easy interfacing to standard fluidic systems. The fabrication route of the transistor-in-a-tube ("tubistor") and the device architecture are shown in Fig. 1, A and B, respectively. The two uniaxial ends of the device act as the inlet and outlet ports, while the central opening is used for the integration of the source-drain electrodes. The gate electrode (i.e., Pt mesh or Ag/AgCl wire) is installed in the extension of the tube close to the inlet port. The 3D channel of the tubistor consists of a porous PEDOT:PSS scaffold formed in situ by the freeze-drying process, as described elsewhere. Briefly, the process involves freezing the aqueous polymer solution at a controlled rate, followed by sublimation of the ice crystals under high-vacuum conditions. This leads to a porous matrix with high surface-to-volume ratio and an extensive 3D network of interconnected pores.

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The mean pore size [estimated from the scanning electron micrographs of neat PEDOT:PSS, PEDOT:PSS/DBSA, PEDOT:PSS/DBSA/collagen, and PEDOT:PSS/DBSA/SWCNT (single-walled carbon nanotube) scaffolds (Fig. 2, A to D). The scaffolds exhibited highly interconnected porous networks, with pore sizes ranging from ~50 to 120 μm. Here, the freeze-drying parameters during the fabrication of the various scaffolds were kept constant (Tc = −50°C, 0.8°C/min). In the case of neat PEDOT:PSS, a more random pore size distribution is apparent, while the inclusion of DBSA improves the homogeneity of pore formation likely due to its surfactant properties, which promote the dispersion of solid parts in the pristine solution.

Variations in the PEDOT:PSS formulation can also alter the electrical and mechanical properties of the resulting scaffolds. As we have shown previously, adding DBSA or collagen in the PEDOT:PSS solution markedly changes the conductivity or the mechanical stiffness, respectively (28). Following this rationale, we proceeded to investigate the effects of different PEDOT:PSS formulations on the electrical performance of the tubistors illustrated in SEM micrographs of neat PEDOT:PSS, PEDOT:PSS/DBSA, PEDOT:PSS/DBSA/collagen, and PEDOT:PSS/DBSA/SWCNT (single-walled carbon nanotube) scaffolds (Fig. 2, A to D). The scaffolds exhibited highly interconnected porous networks, with pore sizes ranging from ~50 to 120 μm. Here, the freeze-drying parameters during the fabrication of the various scaffolds were kept constant (Tc = −50°C, 0.8°C/min). In the case of neat PEDOT:PSS, a more random pore size distribution is apparent, while the inclusion of DBSA improves the homogeneity of pore formation likely due to its surfactant properties, which promote the dispersion of solid parts in the pristine solution.
The presence of collagen in the PEDOT:PSS/DBSA mixture did not seem to induce any macroscale morphological effect on the resulting porous structure. In the case of the SWCNT mixture, wire-like nanodomains protrude slightly from the scaffold surface (Fig. 2D, inset).

More quantifiable changes can be observed in the electrical behavior of the tubistors, namely, the comparative output characteristics and the evolution of transconductance at the given $V_{GS}$ range (Fig. 2, E and F). When used as the channel material, DBSA-based scaffolds exhibited substantial improvement in the electrical performance compared to pristine samples, as confirmed by the transistor characteristics of the corresponding tubistors, consistent with previous reports on DBSA-enhanced conductivity of PEDOT:PSS (25). Specifically, the magnitude of $g_{DS}$ increased about six times with DBSA (from ~0.7 to ~4.2 mA), while the $g_{m}$ increased from 0.95 to 11.2 mS. Although the addition of collagen may provide better biocompatibility and mechanical properties for tissue growth studies, its insulating nature degrades the conductivity of the scaffolds and the overall performance of the tubistors. In contrast, the incorporation of SWCNTs into the PEDOT:PSS/DBSA mixture resulted in tubistors with high transconductance values of 18.7 mS (best recorded performance) (40, 41). Future work will include a more detailed study on the structural, morphological, and electrical properties of PEDOT:PSS/nanomaterial (CNTs, graphene, etc.) scaffolds.

As described by Jimison et al. (42), OECTs can be used for assessing and monitoring the integrity of barrier tissues. In a first attempt to operate a tubistor as an electrochemical transducer for biological events, two different cell lines were seeded on tubular-shaped (length, 4 mm; diameter, 1.5 mm) PEDOT:PSS scaffolds, namely, barrier-forming kidney epithelial cells (MDCKII) and telomerase-immortalized fibroblasts (TIFs). An image of various free-standing PEDOT:PSS scaffolds is shown in Fig. 3A. In this set of experiments, the cell seeding and growth stage took place inside an Eppendorf tube for 3 days. The tissue-containing scaffolds were then inserted inside the tubistor to investigate the effects of cell growth on the electrical performance of the tubistor (Fig. 3B). Fluorescence images (Fig. 3, C and D) taken after 3 days of culture provide evidence of cell accumulation in the scaffold, with somewhat inhomogeneous cell distribution and coverage apparent for both cell types. This is consistent with previous observations of cell growth in PEDOT:PSS scaffolds and can be attributed to the lack of a perfusion system (28). While TIF cells appeared to be spread over the scaffold forming individual cell domains, scaffolds seeded with MDCKII cells exhibited high conformity, with tissue-like morphology at the pore surface, as shown in the magnified image of Fig. 3C.

The adhesion of cells and subsequent tissue formation inside the pores of the scaffold were seen to substantially change the drain current, as shown in the output characteristics of fig. S5. This change is accompanied with a shift toward negative $V_{DS}$, likely due to non-ideal physical contact between electrodes and semiconductor, which results in poor charge injection, especially in the cell-covered scaffolds. The growth of cells in the scaffolds was further found to affect the efficiency of the ionic signal transduction, expressed by the magnitude of $g_{m}$. Comparing the maximum normalized $g_{m}$ values as a function of $V_{GS}$ between the scaffolds containing cells and those without cells, we noticed a decrease of 72 and 60% for MDCKII cell–seeded (Fig. 3E) and TIF cell–seeded (Fig. 3F) scaffolds, respectively. In agreement with previous observations on planar OECTs, the formation of barrier tissue (e.g., MDCKII) on the PEDOT:PSS channel of the OECT changes the transistor performance, i.e., increase...
in response time (τ). This was further confirmed by the pulse characteristics of the tubistors (Fig. 3, G and H). A marked difference in the relative change of the response time between the two cell types can be observed. Specifically, in the case of the MDCKII epithelial cells where ionic transport is hindered by the tight barrier properties, the response time (τ) was found to increase substantially from 1.4 to 3.1 s. In contrast, the TIF cell (non–barrier tissue–forming cell)–seeded devices exhibited a negligible change from 2.0 to 2.6 s.

We posit that this difference is due to additional resistance effects conveyed by the epithelial cells, in contrast to the fibroblast cells. This is supported by previous work screening multiple cell types (barrier-forming versus non–barrier-forming) on 2D planar devices (43). Control experiments carried out without cells in culture medium for 4 days showed only a slight degradation of the device performance, as shown in fig. S6. Specifically, a decrease in the $I_{DS}$ of about 17% was measured, while the corresponding change in the maximum $g_m$ was found to be approximately 18%.

To assess the versatility of our 3D devices, we performed in situ real-time monitoring of cell growth. The fluidic structure of the tubistors promotes the efficient perfusion of the scaffolds by supplying a continuous flow of medium (0.5 μl/min) during cell culture while monitoring the transistor parameters. As discussed earlier, to
render the tubistor biocompatible with long-term electrical measurements, a Pt mesh embedded inside the tubing was used as the gate electrode. A schematic illustration of the experimental setup is shown in Fig. 4A. In this set of experiments, MDCKII cells were cultured inside the tubistor without any pretreatment of the scaffold and imaged after 1 and 2 days (Fig. 4, B and C). A homogeneous spreading and distribution of cells is clearly visible throughout the scaffold, along with extensive tissue formation after 2 days of culture. This observation highlights the important role of a perfusion system when hosting 3D cell cultures. During these 2 days, we could monitor fluctuations in the electrical performance of the devices associated with various cellular growth stages. The output characteristics of the tubistors at certain time points during the cell culture process show a marked drop in the magnitude of $I_{DS}$ ($V_{DS} = -0.6$ V) from $-1.6$ to 0.46 mA after the seeding and during the incubation (1 hour without flow), accompanied with a reduction of the $g_m$ by more than two orders of magnitude (Fig. 4, D and E). We attribute these changes to the high density of the initial cell suspension, which may hamper the injection of charge carriers and the diffusion of ions. After the incubation step, the nonadherent cells were expelled by flowing fresh medium into the system, resulting in a partial recovery of the device performance. Additional experiments were carried out to investigate the initial effect of cell density on the device performance by varying the number of cells seeded into the scaffolds ($4 \times 10^3$ versus $4 \times 10^5$ cells). We could observe a notable difference on the device performance after 1 hour of seeding with the different cell densities. The higher cell density seeded device resulted in a larger decrease in the current magnitude accompanied by a change in the $g_m$ value of $-28\%$ versus a $-16\%$ change for the lower cell density (Fig. S7). The growth of cells in the 3D matrix has a direct effect on the device performance, most likely related to the ionic and channel resistance. Thus, by adjusting the cell number at a given scaffold size, we can tune the sensitivity level of the device to assess the initial stages of cell attachment. During this stage, device-to-device variations may be observed due to the random cell distribution and coverage. Notably, after cell attachment, the devices show a steady behavior, while a gradual decrease over time in the $g_m$ magnitude was observed after $t = 16$ hours of cell culture, as shown in Fig. 4E. The cellular organization inside the scaffolds during the first 48 hours strongly dictates the electrical operation of the devices, with a relative decrease in the maximum $g_m$ value of approximately 82%. Because of the small size of the scaffold and the good perfusion capability of the tubistor, we were able to obtain extensive cell growth after only 2 days of culture. After 44 hours of cell culture, there are no major variations in the $g_m$ indicating that a confluent tissue has been obtained. In situ electrical measurements of an incubated device without cells did not show any significant variation in the $g_m$ value over time, as shown in the control curve (denoted as stars) of Fig. 4E.

We and others have shown that cell adhesion can greatly change the impedance of electrodes on which they are cultured; however, other processes such as barrier formation (typical of epithelial cells such as the MDCKII cells used here) can have additional electrical effects that may be observed. To illustrate the future potential of these devices for continuous toxicology monitoring, we performed preliminary experiments with EGTA, a calcium chelator that disrupts paracellualar junctions, thus impairing the tissue barrier. Confirming the effects of EGTA (100 mM) on our 3D cultured devices, a progressive disruption of the cell barrier was evidenced by a decrease in the time constant $\tau$, as the tight junctions undergo disassembly (Fig. S8A). Specifically, a rapid decrease in the normalized $\tau$ value was observed within the first 15 min, implying compromise of the barrier integrity (Fig. S8B). These findings are in good agreement with our previous studies on the effects of EGTA on paracellular permeability using 2D OECTs (13, 44).

**DISCUSSION**

From the first demonstration of the recording of a leech neuron using Si-based transistor technology (4) to more recent studies such as probing single-cell activity using a tunable 3D sensing nanoscale
field-effect transistor by Tian et al. (45), transistors have proven highly advantageous for cell monitoring. As inherently more biomimetic transistors, due to the organic nature of the material comprising the channel, OECTs have gone from strength to strength in interfacing with cells. In particular, OECTs have been used not only for electroactive cell monitoring but also for monitoring of tissue integrity, with a degree of superiority over the traditional two-electrode format used in electrical impedance sensing of cells (11, 46). Apart from the patch clamp technique (or microneedle-type approaches) to monitor intracellular electrical activity, electrodes for interfacing with cells are themselves almost exclusively 2D, limiting efficiency in electrical measurements of more physiologically relevant 3D tissue structures. Undeniably, 3D cell biology has and will continue to benefit from developments in materials science and biology with sophisticated and physiologically relevant complex models, and so technologies that can adapt to accurately monitor those systems are urgently needed. Hence, our innovative concept of a fully integrated 3D polymer-based transistor in a tube as both the cell tissue support and the active transducer heralds a new path toward truly biomimetic 3D in vitro (bio)electronics. We have successfully addressed challenges from a (micro)electronics standpoint related to stability, electrical performance, and system integration. Our 3D OECT was shown to exhibit remarkably good and temporally stable electrical characteristics and robustness, owing to its design and the in situ formation of the conducting channel. From a materials/functionality standpoint, we have shown the potential to fabricate a wide variety of mesoscale and macroscale geometries, and that by tuning the components in the precursor conducting solution, we can easily adjust both the device performance (i.e., electrical conductivity) and the scaffold properties (i.e., porosity) to fit different purposes. We also found that our devices exhibited stress-dependent electrical behavior owing to the good elastic properties of the PEDOT:PSS scaffolds, as shown in fig. S9. The cumulative compression of the scaffold inside the tubistor led to a gradual increase in the measured drain current possibly due to the establishment of more conducting points. Since this observation was beyond our current application scope, future studies will investigate more thoroughly the capability of our device for pressure sensing.

From a biological standpoint, we have shown excellent compatibility of the 3D conducting scaffolds with two different types of cells both when seeded ex situ and in situ using continuous flow through our integrated fluidic. The integrated fluidic provides greater ease of use and compatibility with biological systems. As expected, the in situ seeding resulted in substantially faster cell adhesion and growth due to the continuous exchange of medium through our perfusion system. The tubistor was also used as the active transducer of cell attachment and growth within the scaffold. As observed, different cell types resulted in different changes both in the steady state and in the transient response. As expected, the barrier-forming cells inhibited the ionic flow to a greater extent and resulted in a more pronounced suppression of the electrical characteristics when compared to cells that are not known to form ionic barriers. In situ dynamic measurements of the tubistor with the barrier-type tissue cells revealed useful information on the timescale of several events, allowing us to postulate the critical stages of cell attachment and growth using the transistor’s gain as the figure of merit for biotransduction. Together, this work opens up a new uncharted area of integrated 3D bioelectronics toward more physiologically relevant in vitro systems. Our future vision includes the development of more complex organoid-type systems including multiple cell types in a compartmentalized manner to study the underlying mechanisms of diseases and aid toward the development of associated therapies.

MATERIALS AND METHODS
Preparation of the 3D scaffolds
Scaffolds were prepared from an aqueous dispersion of PEDOT:PSS (Clevios PH 1000, Heraeus) at a concentration of 1.25 wt %. To enhance the mechanical properties and the stability of the scaffolds in aqueous solution, (3-glycidoxypropyl)-trimethoxysilane (Sigma-Aldrich) was added as a crosslinker (3 wt %). Moreover, to enhance further the conductivity of the scaffolds, we added 0.5% DBSA (Sigma-Aldrich). This was the basic formulation used for the fabrication of the free-standing conducting scaffolds as well as for the tubistors used in the cell studies. Two different additives were used with the basic formulation to evaluate the effects on the electrical behavior of the scaffolds: (i) collagen (0.05 wt %, type I from rat tail) and (ii) SWCNTs (0.5 wt %). Before the freeze-drying process, the PEDOT:PSS dispersion was poured into various molds and tubing systems. For the fabrication of the tubistor, the volume injected inside the tube ranged between 40 and 80 μl depending on the desired dimensions of the system. The samples were then placed in a freeze dryer (Cryotec and Virtis AdVantage 2.0 BenchTop), where they were frozen from 5°C to −50°C at a controlled cooling rate of −0.8°C min⁻¹ (for the standard fabricated devices), at which point the ice phase was sublimed from the scaffolds, as described by Wan et al. (26). After the freeze-drying process, the samples were baked at 70°C for 2 hours. Before device electrical measurements and cell experiments, all scaffolds were rinsed with deionized (DI) water multiple times, and they were then kept in DI water for 24 hours to enable the diffusion of low molecular components out of the structure. For the cell culture experiments, the samples were sterilized using 70% ethanol for about 30 min.

Device preparation
T-shaped tubes (Cole Parmer) were used as hosting structures for the fabrication of the tubistors. For the fabrication of source and drain electrodes, Au-coated (150 nm) Kapton films (120 μm) were used. Using the top opening, the source-drain electrodes were fixed inside the tube using a temporary cylindrical separator to avoid any possible contaminants. Three different gate electrodes were tested with the tubistor devices: (i) Ag/AgCl, (ii) Pt, and (iii) PEDOT: PSS. The different gates were placed inside a tubing extension and sealed. For long-term cell monitoring experiments, we used a Pt mesh electrode. The size of the Pt electrode was substantially larger than the scaffold to ensure efficient gating.

Scaffold and device characterization
The microstructure and morphology of the scaffolds were performed using SEM. SEM ULTRA 55 (Carl Zeiss) was used to evaluate the invasion of cells into the scaffolds. Briefly, cells in the scaffold were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer overnight at 4°C. After extensive washing with PBS, the scaffold was then dehydrated in a graded ethanol series and dried using hexamethyldisilazane.
solution. Last, the sample was coated with 15-nm gold/paladium and analyzed at 5-kV acceleration voltages. The electrical characterization was performed using a solution of 100 mM NaCl in DI water as the electrolyte. The transistor characteristics were measured using a Keithley 2612 source meter, a customized LabVIEW software, and a Keysight B1500A parameter analyzer. The experiments were carried out in ambient atmosphere when no cells were involved, while for the cell studies an incubator at a temperature of 37°C and CO₂ level of 5% was used.

For the estimation of the pore size, SEM image analysis was used. Average pore diameter was measured on the basis of $n = 40$ pores per scaffold.

**Cell culture experiments**

Two cell types were used for the experiments: canine epithelial kidney cells (MDCKII, a gift from F. Luton, Institut de Pharmacologie Moléculaire et Cellulaire, Valbonne) and human TIFs (a gift from E. Van Obberghen-Schilling, Institut de Biologie de Valrose). MDCKII cells were cultured in low-glucose Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum, 2 mM glutamine, penicillin (50 U ml⁻¹), and streptomycin (50 μg ml⁻¹). Fibroblasts were cultured in high-glucose DMEM and supplemented as previously described without glutamine. Once cells were detached from the tissue culture flask using a solution of 0.25% trypsin, cell suspension was centrifuged and supernatant was replaced supplemented as previously described without glutamine. Once cells were detached from the tissue culture flask using a solution of 0.25% trypsin, cell suspension was centrifuged and supernatant was replaced supplemented as previously described without glutamine.

Cell seeding was done right after by dipping the dried scaffold for 2 min. Cell seeding was done right after by dipping the dried scaffold for 2 min. For in situ experiments, the medium was not removed to prevent any bubble formation inside the device, so cells were directly injected into the scaffold using the fluidic tubing at a velocity of 1.5 l/min. For in situ experiments, the medium was not removed to prevent any bubble formation inside the device, so cells were directly injected into the scaffold using the fluidic tubing at a velocity of 1.5 l/min. For the cell adhesion experiments, two different cell suspensions were prepared: 5 × 10⁶ and 5 × 10⁴ cells/ml. A volume of 80 μl of 0.4% trypan blue. Cells were counted using a glass hemocytometer and resuspended to prepare the desired cell concentration. Before cell seeding, scaffolds were kept submerged in cell medium for 2 hours at 37°C, allowing protein adhesion.

For the free-standing scaffold experiment, the medium was completely removed from the scaffold by placing it onto an absorber for 2 min. Cell seeding was done right after by dipping the dried scaffold into a cell suspension (MDCKII or TIF; 5 × 10⁶ cells/ml), allowing cell penetration by capillarity forces. Then, the scaffold was kept at 37°C for 1 hour, allowing cell attachment and spread before changing the medium to remove nonattached cells. Cell culture maintenance was done by placing the scaffold into an Eppendorf tube filled with the medium for up to 3 days.

For in situ experiments, the medium was not removed to prevent any bubble formation inside the device, so cells were directly injected into the scaffold using the fluidic tubing at a velocity of 1.5 l/min. Cell culture maintenance was done using cell medium supplemented with 5 mM Hepes and a continuous flow rate of 0.5 μl/min for 2 days.

For the adhesion experiments, two different cell suspensions were prepared: 5 × 10⁶ and 5 × 10⁴ cells/ml. A volume of 80 μl was injected inside the scaffold device, resulting in 4 × 10⁵ and 4 × 10³ cells, respectively. The electrical measurements were carried out before cell seeding and after incubating the devices for 1 hour at 37°C.

**Immunofluorescence staining**

MDCKII and TIF cells were fixed in 4% paraformaldehyde for 20 min at room temperature. The scaffolds were washed extensively with PBS and incubated with rhodamine phalloidin (Sigma) for 30 min to label actin filament. Fluorescence images of the scaffolds were obtained using an epifluorescence/confocal microscope (Axio Observer Z1 LSM 800, Zeiss).

**EGTA experiments**

MDCKII cells were seeded inside the scaffold devices and incubated to grow for 3 days. After stabilization of the devices for several minutes, we recorded the electrical signal (transient current response) to investigate the effects of EGTA addition. Specifically, a 100 mM solution of EGTA was diluted in cell medium (Alfa Aesar EGTA 0.5 M aqueous solution, J60767) and injected into the scaffold through the fluidic circuit in a steady manner. The transistor recording was carried out for several minutes after EGTA injection. The following parameters were used during the transient response measurements: V_DS = −0.3 V, V_CS = 0.3 V, on time t = 15 s. Data analysis was performed to fit the time constant for each individual pulse (13). Control experiments were carried out by exposing a cell-free scaffold to the EGTA solution for a certain period of time, as shown in the normalized data of fig. S8C.

**SUPPLEMENTARY MATERIALS**

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/4/10/eaat4253/DC1

Fig. S1. Freeze-drying process for the fabrication of PEDOT:PSS scaffolds.

Fig. S2. Transient response of the tubistor.

Fig. S3. Effect of gate electrode on the electrical performance of the tubistor.

Fig. S4. Effect of pore size on the electrical performances of the tubistor devices.

Fig. S5. Monitoring the effects of 3D cell cultures on the steady-state characteristics of the transistor.

Fig. S6. Long-term stability of the tubistor in the presence of cell culture medium.

Fig. S7. Monitoring the effects of cell density during the initial cell attachment stage.

Fig. S8. Effect of EGTA on the barrier function of cells cultured in the tubistor.

Fig. S9. Effect of the compression strain on the electrical properties of the tubistors.

**REFERENCES AND NOTES**


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Author contributions: C.P. conceived, executed, and analyzed results of experiments. M.P.F. did cell culture and SEM. D.I. aided with SEM and scaffold characterization. L.T. prepared and characterized SWCNTs. S.I. aided with initial concept and tubistor preparation. R.M.O. conceived and directed research on the paper. The manuscript was written and edited by C.P. and R.M.O. Competing interests: C.P., S.I., and R.M.O. are inventors on a patent related to this work filed with the French patent authority (application no. FR 1758683, submitted on 20 September 2017). All other authors declare that they have no competing interests.

Data and materials availability: All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Additional data related to this paper may be requested from the authors.

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