A portable cell-based impedance sensor for toxicity testing of drinking water†

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A major limitation to using mammalian cell-based biosensors for field testing of drinking water samples is the difficulty of maintaining cell viability and sterility without an on-site cell culture facility. This paper describes a portable automated bench-top mammalian cell-based toxicity sensor that incorporates enclosed fluidic biochips containing endothelial cells monitored by Electric Cell-substrate Impedance Sensing (ECIS) technology. Long-term maintenance of cells on the biochips is made possible by using a compact, self-contained disposable media delivery system. The toxicity sensor monitors changes in impedance of cell monolayers on the biochips after the introduction of water samples. The fluidic biochip includes an ECIS electronic layer and a polycarbonate channel layer, which together reduce initial impedance disturbances seen in commercially available open well ECIS chips caused by the mechanics of pipetting while maintaining the ability of the cells to respond to toxicants. A curve discrimination program was developed that compares impedance values over time between the control and treatment channels on the fluidic biochip and determines if they are significantly different. Toxicant responses of bovine pulmonary artery endothelial cells grown on fluidic biochips are similar to cells on commercially-available open well chips, and these cells can be maintained in the toxicity sensor device for at least nine days using an automated media delivery system. Longer-term cell storage is possible; bovine lung microvessel endothelial cells survive for up to four months on the fluidic biochips and remain responsive to a model toxicant. This is the first demonstration of a portable bench top system capable of both supporting cell health over extended periods of time and obtaining impedance measurements from endothelial cell monolayers after toxicant exposure.

Introduction

Protection of drinking water supplies from chemical contaminants can be enhanced by the use of toxicity sensors. Living cell sensors can indicate the presence of a broad range of chemicals (including unknown agents) that cause a toxic response, whereas analyte-specific sensors can only quantify and identify specific chemicals. Although toxicity sensors using enzymes or bacteria are presently in use at some water utilities, the use of mammalian cells as sensors is far less common, in spite of the potential relevance of these cells to human physiology.[1–3] One major issue with using mammalian cells in toxicity sensors is the difficulty of maintaining cell viability under field conditions for extended periods of time until they are needed for water testing.[4] This problem has been encountered in previously-developed portable toxicity sensors using cardiomyocytes and neurons.[5–7]

Here we propose a portable cell-based toxicity sensor that measures changes in the impedance of mammalian endothelial cell monolayers. The sensor device is suitable for field use and capable of maintaining sensor cells for extended periods of time. Endothelial cells are well-suited for toxicity sensor applications because of their central location in many organs. Many chemical toxicants end up in the blood stream independent of their primary route of exposure (respiratory tract, gastrointestinal tract, skin, eyes, *etc.*); they can then spread throughout the body and cause secondary sites of injury. Because endothelial cells organize *in vitro* to form a restrictive barrier under normal conditions, several devices have been used to measure the electrical resistance of endothelial cell monolayers. As a versatile and noninvasive tool, ECIS has been able to provide quantitative information with respect to cell morphological changes, cell movements, and alterations in cellular function under various drug, chemical, and biological treatments.[8–16] Although this system has been used for several years to assess cell monolayer integrity after exposure to a variety of agents, to date this system has not been exploited for development as a portable biosensor.

Previous work has shown that using ECIS to monitor the impedance of endothelial monolayers provides a sensitive measure of toxicity for a wide range of chemical toxicants.[17] Also, previous research efforts have demonstrated that cellular impedance is an accurate metric for determining cell viability and thus appropriate for determining cell monolayer health over time.[18] However, these measurements require on-site cell culture facilities as well as equipment and personnel to read the impedance of the cells and to maintain the correct environment for the

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cells. A flow chamber that integrates with the ECIS technology has been developed to allow cell impedance measurements to be taken under flow conditions, but the system still requires substantial supporting equipment and trained personnel to operate.[19] The absence of means for automated cell maintenance precludes the use of this instrumentation in field environmental monitoring applications.

Our goal was to develop a field-portable cell-based impedance biosensor by integrating the ECIS technology with an enclosed fluidic biochip and an automated cell maintenance system that could monitor the impedance of endothelial monolayers after water sample exposure. We developed maintenance protocols that offer longer and more practical storage conditions for the cells. Dedicated statistical software was also created to automatically and accurately detect the presence of toxicants. This is the first demonstration of a portable bench-top system capable of both supporting cell health over extended periods of time and obtaining impedance measurements from endothelial cell monolayers after toxicant exposure.

Experimental

Fluidic biochips

The fluidic biochips (shown in Fig. 1A) were made from two components, a polycarbonate fluidic layer and an ECIS electronic layer. The fluidic layer was designed with two channels, one as a reference channel (control) and one for the water sample under test. The dimensions of the channel were improved to minimize resistance to current flow between the working and counter electrodes. The second component, the ECIS electronic layer, was custom designed and manufactured by Applied BioPhysics (www.biophysics.com; Troy, NY) for the current application. The ECIS electrode pads were re-designed to a rectangular shape from the original circular shape to allow narrower channels on the fluidic layer. Each of the two channels has four electrode pads to allow four impedance readings of the control fluid and four impedance readings of the test fluid for each chip analyzed. Each electrode pad contains 10 working electrodes, which are each 250 μm in diameter. The counter electrode wraps around the working electrodes to insure even electron flow. The ECIS electronics averages the impedance values obtained from each working electrode and displays one value for the entire electrode pad. Fig. 1A shows a picture of the assembled fluidic biochip, with the inset showing one electrode pad in the fluidic channel. This design maximizes the surface area of the counter electrode to the working electrodes, so the total impedance of the system is dominated by the impedance of the working electrodes. The path length from the working electrodes to the counter electrode was minimized to prevent the electrical resistance of the fluid from becoming significant.

Sterile fluidic biochips were seeded with bovine pulmonary artery endothelial cells (BPAECs) or bovine lung microvessel endothelial cells (BLMVECs) and were allowed to develop into a confluent monolayer. Biochips that contained culture medium and no cells had impedance values of 300–400 ohms; impedance values increased to 900–1000 ohms for the BPAECs and 1800 to 2000 ohms for the BLMVECs when confluent monolayers were present in the channels. These impedances are comparable to values previously obtained with the same cell lines in the open well ECIS chips,[17] indicating that the fluidic biochips could support normal endothelial monolayer formation. Sterilized fluidic biochips, ready for cell seeding or pre-seeded with cells, are commercially available (Agave BioSystems, Ithaca, NY).

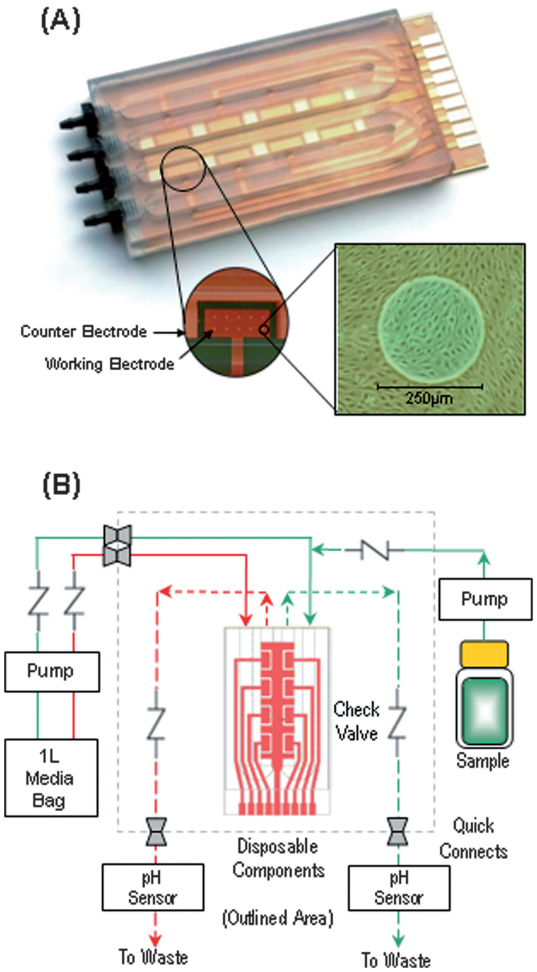

Fig. 1 (A) Custom ECIS fluidic biochip available from Agave Biosystems Inc. (Ithaca, NY). The fluidic layer is attached to the custom ECIS electronic layer. The inset shows one electrode pad which contains a large counter electrode and 10 working electrodes. (B) Fluidic pathway used in automated bench top instrument. Outlined area represents components that are disposed of after test run.

Cell culture and seeding in fluidic biochips

BPAECs and BLMVECs were obtained from VEC Technologies (Rensselaer, NY). The cells were cultured under standard conditions of 37 °C and 5% CO_2 and were used for biochip seeding at passages 5–11. BPAECs were cultured in Minimum Essential Medium (MEM; Invitrogen, Carlsbad, CA) with 20% fetal bovine serum (FBS; Invitrogen). For toxicant exposures, MEM was used supplemented with 0.5% bovine serum albumin (BSA; Sigma-Aldrich, St. Louis, MO) instead of 20% FBS. BLMVECs were cultured in MCDB-131C complete media with 10% FBS (VEC Technologies) and were exposed to chemicals in serum-free MCDB-131 medium (Sigma-Aldrich, St. Louis, MO).

Prior to being seeded with cells, the fluidic biochips were precoated with 0.2% gelatin solution in 0.15 M NaCl for 1 hour in order to facilitate cell attachment and the formation of a continuous endothelial monolayer. The cells were then injected into the fluidic biochip using a sterile syringe. A concentration of 5.0×10^5 cells/ml was used for the BPAEC biochips and 2.5×10^5 cells/ml for the BLMVEC biochips. Cell monolayers were allowed to grow on the biochips in a tissue culture incubator (37 °C and 5% CO_2) (three days for the BPAECs and seven days for the BLMVECs) prior to being used for toxicity or longevity tests.

Fluidic biochip evaluations: control variability

The performance of the fluidic biochips was evaluated through comparison of cell impedance responses with commercially available open well ECIS chips (8W10E; Applied BioPhysics, Inc., Troy, NY). Both control chip variability and responses to toxic chemicals were tested. For control chip testing, variability between duplicate channels on the fluidic biochips, between different fluidic biochips, and between open well chips was evaluated at times 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, and 55 minutes after the start of the test. Four fluidic biochip and four open well chips were tested.

To compare the chip-to-chip variability of fluidic biochips and open well chips, we tested the null hypothesis of equal variability, H_0: $\sigma^2_{c:fl} = \sigma^2_{c:ow}$, against the alternative hypothesis of lower variability among fluidic biochips than among open well chips, H_a: $\sigma^2_{c:fl} < \sigma^2_{c:ow}$. We performed an F test for equality of variance among the mean normalized impedances for the two types of chips. We computed $s^2_{c:fl}$ and $s^2_{c:ow}$, the sample variances of the mean normalized impedances from the 4 chips of each type, from the data. The test compares these values, rejecting the null hypothesis of equal variability among chips if the test statistic $F_a = s^2_{c:fl}/s^2_{c:ow} < F_{0.05;3,3} = 0.1078$. To compare the within-chip variability of the electrode pads on fluidic biochips and those on open well chips, we tested the null hypothesis of equal variability, H_0: $\sigma^2_{p:fl} = \sigma^2_{p:ow}$, against the alternative hypothesis of lower variability among the pads on a fluidic biochip than among those on an open well chip, H_a: $\sigma^2_{p:fl} < \sigma^2_{p:ow}$. We performed an F test for equality of variance for the two types of chips among the normalized impedances from the pads within a chip. We computed $s^2_{p:fl}$ and $s^2_{p:ow}$, the estimated variability among pads on a chip of each type, from the data. The test compares these values, rejecting the null hypothesis of equal variability among pads within a chip if $F_w = s^2_{p:fl}/s^2_{p:ow} < F_{0.05;28,28} = 0.5313$.

Fluidic biochip evaluations: toxicant testing

For comparisons of toxicant responses between fluidic biochips and open well chips, exposures were conducted with several chemicals at concentrations that caused impedance responses in BPAEC cells in open well chips: phenol – 1.1 mM; 1,1,2-trichloroethane – 2.5 mM; potassium cyanide – 0.35 mM; lindane – 0.1 mM; and pentachlorophenate (PCP) – 0.38 mM. All the test chemicals except PCP were obtained from Sigma-Aldrich (St. Louis, MO) and were prepared in distilled water. PCP (Mallinckrodt Baker, Phillipsburg, NJ) was prepared from the sodium salt of pentachlorophenol in 2 mM phosphate buffer and pH adjusted to 7.5.

Prior to evaluation on BPAEC cells, each test chemical was diluted 1 : 1 with twice concentrated culture medium (powdered version of MEM used to culture the cells with 0.5% BSA; Invitrogen). The pH of the diluted chemical toxicants was adjusted to 7.4 and the temperature of the test solutions was brought to 37 °C prior to cell testing by incubating samples in an incubator for 30 minutes. One hour prior to chemical testing in the fluidic biochip, the serum containing MEM was removed from both channels and MEM with 0.5% BSA was added. After one hour, the impedance values were measured and recorded in each channel, then the medium was removed and either 5 mL of the test chemical in MEM + 0.5% BSA or 5 mL of MEM + 0.5% BSA alone (control channel) were added to the channels using sterile syringes. Impedance values were monitored in each channel every minute for approximately 60 minutes. The impedance readings in all experiments were represented as normalized impedance, which is the impedance value at each time point divided by the initial impedance value (prior to chemical exposure) on each electrode. The starting impedance values on each electrode varied due to inherent variability of the cells covering the electrodes. Normalizing the data allowed for a more uniform comparison of impedance values between electrodes and, subsequently, between experiments.

For chemical testing in the prototype toxicity sensor, the media bag was filled with MEM + 0.5% BSA, and a fluidic biochip with a confluent monolayer of BPAECs was assembled into the instrument in a laminar flow hood to allow sterile connections between the media bag, fluidic biochip, and tubing of the fluidic system. Once the system was assembled, the prototype was removed from the hood and testing was done on the lab bench. To establish a baseline response, impedance values were taken every minute over a period of approximately one hour to evaluate the stability of the cell layer in the portable instrument, at which time a toxicant was introduced (0.38 mM PCP). Impedance values were monitored over several hours to track the cellular response over time.

Fluidic biochip evaluations: cell longevity

Longevity of the BPAEC cells was evaluated using fluidic biochips in the prototype toxicity sensor. A fluidic biochip containing a confluent monolayer of BPAECs was placed in the prototype. The media bag was aseptically filled with MEM + 20% FBS and the pumps were programmed to pump at 1 mL/min for 15 minutes every six hours. Impedance readings were then taken periodically over a nine-day period to evaluate whether impedances would remain stable (defined as by a decrease of no more than 20% from initial values and no substantial changes in cell morphology, as visualized by phase contrast microscopy). Previous research efforts have demonstrated that cellular impedance is an accurate metric for determining cell viability and thus appropriate for determining monolayer health over time.[18] In addition, pH values were monitored to determine whether the CO_2 impermeable fluidic system was successful in maintaining the pH of the medium over long periods of time.

The BLMVECs were used for longer-term longevity studies. After two weeks or more under *in vitro* conditions, a small percentage of the BPAECs kept dividing and formed a layer on top of the existing monolayer, which could decrease the detection

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sensitivity of the system and prevent long-term storage of the cells by overgrowing the chip. Subsequent testing showed that BLMVECs form a quiescent endothelial monolayer in the fluidic biochips that is stable for much longer periods. Fluidic biochips were seeded with BLMVECs, held in a 37 °C incubator with 5% CO_2, and were provided fresh medium three times per week. At 1, 2, 4, 8, and 16 weeks post-seeding, impedance readings were recorded and cells were challenged for one hour with PCP (0.038 mM). Serum-free MCDB-131 medium was used for PCP exposures. A curve discrimination program (described below) evaluated whether the PCP caused significant reductions in impedance.

Toxicity sensor design

Hardware. A portable instrument was designed and fabricated that included the following functions: pumping of cell culture medium and chemical toxicants into fluidic biochips, monitoring the pH of the effluent medium flowing from the fluidic biochip, maintaining the fluid temperature and sterility of the culture medium, and making continuous impedance measurements of the cells during exposure to the chemical toxicants. This portable instrument supports both cell health and chemical testing in the fluidic biochip. As a result of this research effort both the portable instrument and disposable fluidic biochips are commercially available through Agave BioSystems (www.agavebio.com). Some of the basic design characteristics of the instrument that allow functionality in a field environment are outlined below.

To maintain the health of the cells in the fluidic biochip and to deliver chemical toxicants, a fluid delivery system was designed (shown in Fig. 1B). The fluidic system was constructed with disposable plastic components that had low CO_2 gas transmission rates, which allowed the pH of pre-gassed medium to remain at 7.4 without the use of exogenously supplied CO_2 gas. The fluidic system was designed to be a plug-and-play unit for ease of use and maintenance of sterility. The area outlined in Fig. 1B represents the disposable portion of the fluidic system that is replaced after completion of test runs. A 1 liter media bag can support 8–10 fluidic biochips for at least 20 days if cells require daily feeding. Pumping of fluids through the system was accomplished *via* a peristaltic pump, which was ideal for this application because the pump mechanics did not contact the culture medium in the fluidic system. For feeding, the pump was programmed to run for 15 minutes to allow adequate flushing of the fluidic channels. The flow rate of the pumps was set to 1 ml/min, which corresponds to shear stress of less than 1 dyn/cm^2. Endothelial monolayer electrical impedance can change substantially when a shear stress of 10 dynes/cm^2 is applied.[19] Small changes in impedance are observed when the cells are periodically fed, but these changes are transient and cease when the feeding cycle ends.

During maintenance mode (when chemical toxicants are not being tested), the pump on the left (Fig. 1B) is periodically activated to feed the cells in the fluidic channel. When a water sample is ready to test, it is mixed with concentrated media (either 2× media or powdered media) to correct the osmolarity of the water sample, and then it is injected into the sample channel. The osmolarity corrected water sample can also be placed in the sample reservoir, and the pump on the right can be used to automatically pump the test sample into the biochip. The control channel on the biochip can either receive media from the media bag, or concentrated media can be mixed with water and then introduced into the channel by a syringe.

The instrument components were positioned in a durable portable enclosure, utilizing a custom baseplate and instrument panel (Fig. 2). The fluidic bag containing cell medium was mounted in the lid of the case. The two peristaltic pumps and fluidic biochip holder were located in custom cutouts on the instrument top panel, which is easily accessible when the case lid is opened. The custom fluidic biochip holder was made out of black anodized aluminum with an integrated thermoelectric cooler to maintain the temperature of the cells at 37 °C in field conditions. This component also allowed for electrical and fluidic connections in a field environment, as well as providing a means for securing the fluidic biochip. The power cord, USB input, power on switch, and indicator light-emitting diode (LED) were also positioned on the instrument top panel. A custom cutout was fabricated to hold a 20 mL sample vial. Samples can be introduced either by standard sterile syringe or by filling the supplied sample reservoir. The final items located on the custom top panel were two stainless steel, panel mount quick connects that join the output channels of the biochip to the bottom of the instrument, where two pH flow cells were mounted to measure the pH of the effluent before the waste container. The other items positioned on the instrument baseplate (below the top panel) were the DC power supply and circuit card stack (microcontroller, thermo-electric cooler (TEC) control, impedance measurement, communication interface, and auxiliary card). The electronics required to take impedance readings of the cells were purchased from Applied BioPhysics (Troy, NY) and integrated into the prototype. In the current study, a 15 Khz frequency was

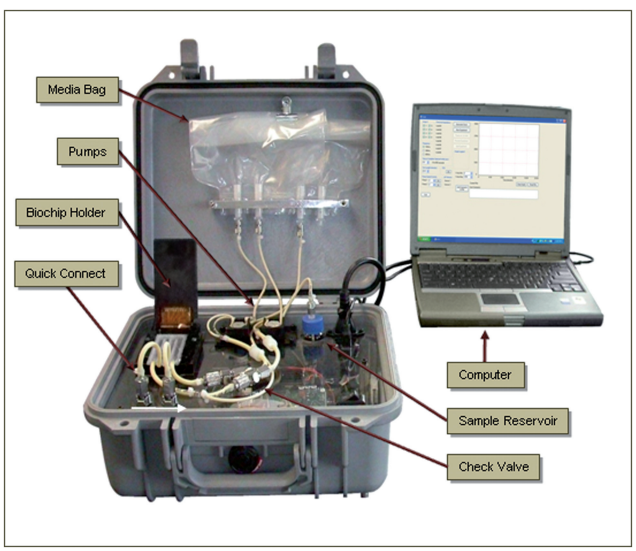

Fig. 2 Portable mammalian cell-based toxicity sensor with automated cell maintenance and sample delivery functions. The device is shown connected to a control computer with graphical user interface (GUI) shown.

used to assess impedance of the cell covered gold electrodes, as described in ref. 20.

Software. The firmware and user interface were developed using Visual Studio (Microsoft Corporation). The software allows the user to control the two pump speeds, on/off time of the pumps, TEC on/off (37 °C), modulation frequency of the impedance measurement signal, total test time, and time between impedance measurements. The software also reports the pH of the two channels as well as the temperature of the fluidic biochip holder. Microcontroller firmware was written to take commands from the user interface software to control the TECs, pumps, pH sensor, and cell impedance circuit. After placing the fluidic biochip and toxicant sample in the instrument, the user selects the total measurement time and pump speeds, adds any experimental notes, and then presses "Begin." The software and firmware automatically sets and controls the temperature, begins the impedance measurement sequence on the eight electrodes, and then saves the data to a file.

To determine when the impedance values generated by toxicant-exposed cells differed significantly from the impedance values generated by the control cells, a curve discrimination program was written using MATLAB (The MathWorks, Inc., Natick, MA). This program analyzed impedance data from control and toxicant-exposed cells for each minute in the exposure period of approximately 60 minutes. The overall difference between the two groups of curves was measured at each time point, and the difference between their averages was assessed relative to the within-group variability. A confidence level of 95% ($P < 0.05$) was used to establish statistical significance. Functional data analysis techniques were used to extend the standard analysis of variance (ANOVA) approach for a single time point to analysis of a curve consisting of approximately 60 points.[21,22] A moving window approach, in which analysis was limited to selected sub-intervals of the full hour, was used to improve effectiveness by concentrating attention on those regions where the difference between non-control and control curves was greatest.

Results and discussion

Variability of fluidic biochip and open well chip controls

Controls for the fluidic biochip exhibit less variability within experiments and between experiments when compared to the open well chips (Fig. 3). This pattern was confirmed by statistical analysis. The results of the test comparing the chip-to-chip variability of fluidic biochips and open well chips led consistently to rejection of equal variability. At times $t = 5$, 10, 15, 20, 25, 30, 35, 40, 45, 50, and 55 minutes, all F_a values were well below the critical value of 0.1078, leading to rejection of the null hypothesis of equal variability among fluidic biochips and among open well chips; the data supported the alternative hypothesis of greater variability among open well chips.

The results of the test comparing the within-chip variability of the pads on fluidic biochips and those on open well chips led to rejection of equal variability after an initial period. At times $t =$ 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, and 55 minutes, we obtained F_w = 1.0143, 1.4115, 1.0472, 0.7778, 0.5996, 0.5200, 0.3861, 0.3703, 0.3425, 0.2865, and 0.3201. The results in the initial period are explained by the fact that the data from each pad were normalized by dividing the observed impedance by the value at time $t = 0$; thus the data values from each pad began with the normalized value of 1 at time $t = 0$, and there was an initial period before the data values diverged sufficiently for differences among pads to be detected. As expected, there is less within-chip variability for the fluidic biochips, whereby samples are introduced to the channels using sterile syringes than in the open well chips, where samples are pipetted onto the electrodes.

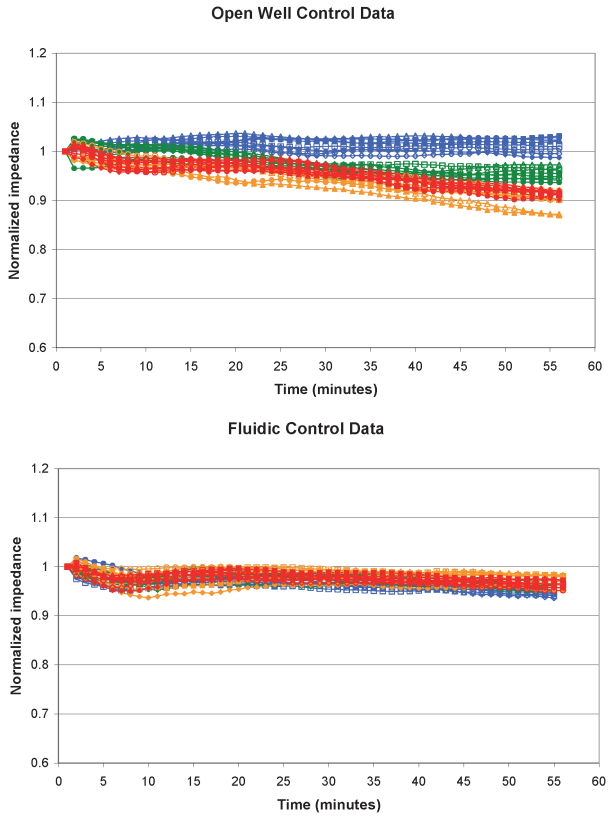

Fig. 3 Normalized impedance of confluent BPAEC monolayers in either open well chip or fluidic biochip after control media added. Each tracing is the normalized impedance from one electrode pad, and 8 pads per chip were analyzed, with 4 chips (open well or fluidic) per graph.

Toxicant responses of fluidic biochips and open well chips

The BPAECs responded significantly to the presence of 0.1 mM lindane in both the open well and fluidic biochip formats (Fig. 4). The initial impedance disturbance frequently observed in the open well chip is seen less frequently in the fluidic biochip. In similar tests with phenol (1.1 mM), 1,1,2-trichloroethane (2.5 mM), and potassium cyanide (0.35 mM), toxicant-treated cells were statistically different than control treatments (see ESI†).

Toxicity sensor performance

The subsystems of the prototype toxicity sensor were found to be functional and correctly integrated. Subsystems that were tested include CO_2 impermeable media bags, fluidics, peristaltic pumps,

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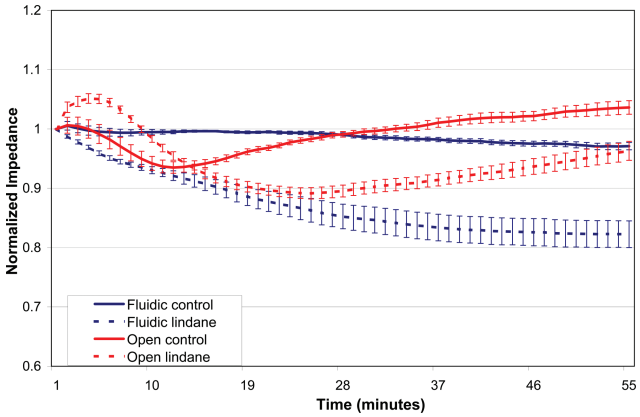

Fig. 4 Normalized impedance of BPAEC monolayers exposed to lindane (0.1 mM) in either the open well chip or the fluidic biochip. The data are represented as the mean ± SEM from two experiments with eight wells per group.

and the TEC controller. The CO_2 impermeability of the media bag was tested by filling the bag with 5% CO_2 equilibrated medium and incubating the capped bag on the bench top for 2 weeks. At the end of this period, the pH of the media was still 7.4, indicating that the 5% CO_2 environment was maintained within the bag over an extended time period. The temperature of the TEC heated array holder was validated using an Omega HH309 digital data logger thermometer (Omega Engineering Inc. Stamford, CT). The average temperature measured over a one hour period was 37 °C (with a range of ±0.5 °C). The peristaltic pump flow rates were controlled by selecting a numerical value from 1 to 10 in the GUI software that correlated to a control voltage for the DC pump motor. For all tests, the numerical value for the pump flow control was one, which equated approximately to a fluid speed of 1 ml per min. The graphical user interface (GUI) of the ECIS prototype was tested and validated. Each subsystem was controlled by software that allowed the user to control the pump speeds, on/off time of the pumps, TEC on/off, modulation frequency of the impedance measurement signal, total test time, and time between impedance measurements. All software was functional and correctly regulated each subsystem.

Fluidic biochip toxicant response in the toxicity sensor

Fluidic biochip performance in the prototype toxicity sensor is shown in Fig. 5. When maintained on medium alone, impedance values were constant over the one hour period prior to toxicant exposure. Introduction of 0.38 mM PCP caused a substantial and highly significant decrease in impedance compared to the control values. Collectively, these data indicate that the portable prototype can support cell health and accurately detect chemical toxicants. Toxicant studies were performed using both 2× liquid medium and powdered medium to demonstrate the ability to test whole water samples. No differences in toxicant sensitivity were noted between the two testing media. The initial disturbance in impedance after the samples were added by syringe injection is most likely due to shear-stress induced changes in impedance that were previously characterized.[19]

Maintenance of cells in portable prototype over extended time periods

For a cell-based biosensor to be usable in the field, the cells in the fluidic biochip must be stable during transport and before use. Impedance and pH readings were taken periodically over a nine-day period from a fluidic biochip containing BPAECs supported in the portable prototype. The data summarized in Table 1 shows that over the monitoring period, the impedances remained stable, indicating that healthy monolayers can be maintained in the portable prototype over extended periods.

A bubble in the second channel impeded fluid flow on day 5, causing a temporary drop in impedance for electrodes 5–8. Once the bubble was flushed out of the channel, the impedance levels returned to the initial values or higher. The data also show that the pH of the medium sampled from the exit port of the fluidic biochip was in the neutral range over the nine-day monitoring period, indicating that the CO_2 impermeable fluidic system was

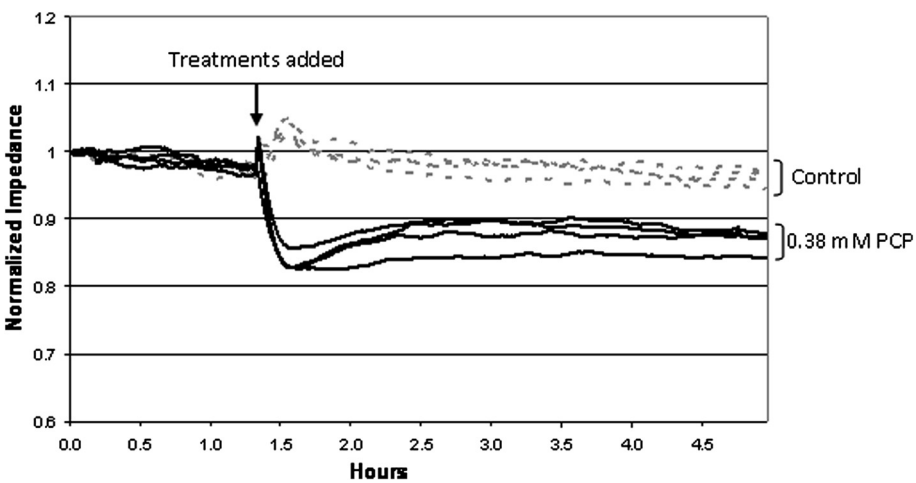

Fig. 5 Response of BPAECs on the fluidic biochip in the prototype toxicity sensor. After 1.3 hours, medium alone was added into one channel and the toxicant (0.38 mM PCP) was added into the other channel of the chip. Each tracing represents the normalized impedance reading from one electrode pad; there are four electrode pads in each channel of the fluidic biochip.

Table 1 Impedance values (ohms) of BPAECs in a fluidic biochip supported by the portable prototype over a 9-day period

		Electrode position on fluidic chip							
Days	pH	1	2	3	4	5	6	7	8
1	7.5	890	987	949	898	876	950	961	934
2	7.0	878	928	870	922	887	934	895	908
3	7.1	862	924	929	981	867	932	962	956
4	7.1	907	987	949	981	858	943	951	994
5	7.1	913	1003	982	1015	647	796	830	917
8	7.5	947	1020	1003	1001	761	847	854	854
9	7.0	968	1038	1024	1005	863	949	946	963

successful in maintaining the pH of the media over long periods of time. Some variability was noted in the pH readings depending on the length of time since last feeding.

Chemical toxicant response in fluidic biochip after long-term storage

The BLMVECs were used for long-term storage studies. The data in Fig. 6 shows that even after the cells are stored for 16 weeks, PCP still caused a significant change in impedance when compared to controls not exposed to PCP. Although the differences between the control and PCP-treated channels decreased as the age of the chips increased, the differences were still statistically significant, as determined by the curve discrimination software.

The 9-day survival of BPAEC cells in the portable toxicity sensor and the up to 16-week survival of BLMVEC cells on the fluidic biochips represents a substantial improvement over other published portable toxicity sensor systems; DeBusschere and Kovacs showed support of functional cardiomyocytes for 8 days, while Pancrazio *et al.* maintained functional cultured neuronal networks for 2 days.[5,7]

Conclusions

The creation of the fluidic biochip was a critical step in developing a portable cell-based toxicity sensor that allows long-term maintenance of mammalian cells without the need of a separate cell culture facility or support technicians. The fluidic biochip allowed a closed fluidic system to be designed that automatically feeds the cells, prevents contamination of the cell monolayers, and contains biological and chemical wastes generated during storage and testing. The use of CO_2 impermeable components eliminated the need for bulky CO_2 canisters otherwise required to maintain cell culture medium pH. Toxicant sensitivity of cells held on the fluidic biochip is similar to that of cells maintained on commercially-available open well ECIS chips, with less impedance variability caused by sample introduction into open well chips. To improve the suitability of the ECIS system for field use, the current prototype toxicity sensor is now being expanded to

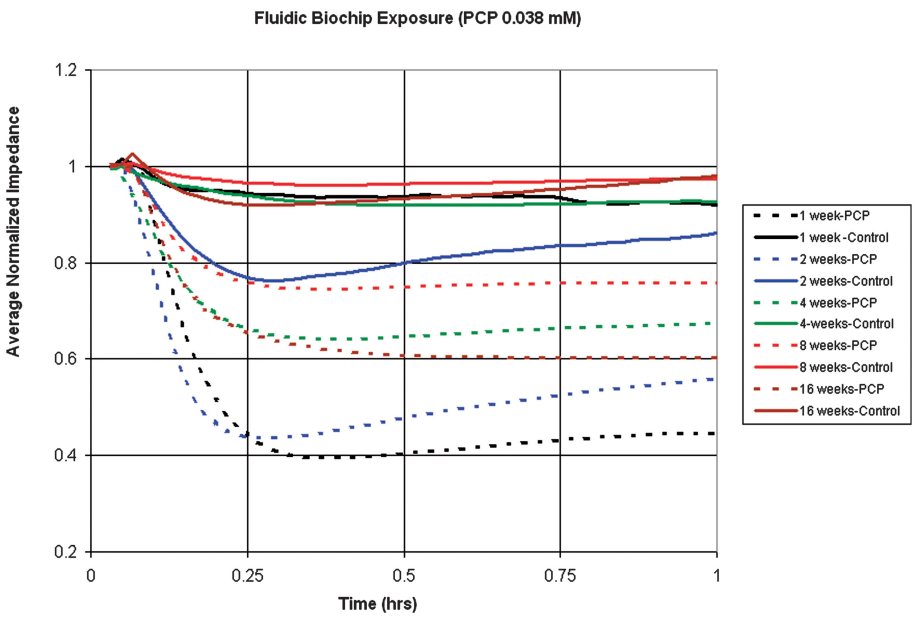

Fig. 6 Average normalized impedance of confluent BLMVECs on fluidic biochips after addition of 0.038 mM PCP or control medium. Each tracing represents the average normalized impedance from 2 replicate fluidic biochip channels (4 electrode pads per channel). Biochips were either 1, 2, 4, 8, or 16 weeks post-seeding at the time of PCP treatment.

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accommodate multiple fluidic biochips that can be held for 30 days or more prior to testing.

We anticipate that this toxicity sensor will be useful for evaluating samples at water utilities and other sites where field toxicity testing with mammalian cells is desired. States *et al.*[1] note that toxicity sensor assays are presumptive tests to be used not as a substitute for a standard analytical test, but "to provide information for rapid hazard assessment and timely selection of operational and public health responses". Because this toxicity sensor can rapidly screen drinking water samples for chemical-related toxicity, it can be used as a toxicity-based indicator of the potential presence of a wide range of toxic chemicals, leading to further analytical evaluation to determine the specific chemical causing the response. Since comprehensive analyte-specific evaluations are too expensive for every day water utility use (several thousand dollars for both inorganic and organic constituents), the more frequent use of a relatively low-cost toxicity sensor test can be a cost effective way to improve the odds that potential chemical contamination is discovered before adverse consequences are manifested. The current cost for an unseeded disposable fluidic biochip is around $60. It is anticipated that with continued technological advancement and development of the technology that the costs may be further reduced.

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