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Volatile Organic Compound Specific Detection by Electrochemical Signals Using a Cell-Based Sensor

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A cell-based *in vitro* exposure system was developed to determine whether oxidative stress plays a role in the cytotoxic effects of volatile organic compounds (VOCs) such as benzene, toluene, xylene, and chlorobenzene, using human epithelial HeLa cells. Thin films based on cysteine-terminated synthetic oligopeptides were fabricated for immobilization of the HeLa cells on a gold (Au) substrate. In addition, an immobilized cell-based sensor was applied to the electrochemical detection of the VOCs. Layer formation and immobilization of the cells were investigated with surface plasmon resonance (SPR), cyclic voltammetry (CV), and electrochemical impedance spectroscopy (EIS). The adhered living cells were exposed to VOCs; this caused a change in the SPR angle and the VOC-specific electrochemical signal. In addition, VOC toxicity was found to correlate with the degree of nitric oxide (NO) generation and EIS. The primary reason for the marked increase in impedance was the change of aqueous electrolyte composition as a result of cell responses. The p53 and NF-κB downregulation were closely related to the magnitude of growth inhibition associated with increasing concentrations of each VOC. Therefore, the proposed cell immobilization method, using a self-assembly technique and VOC-specific electrochemical signals, can be applied to construct a cell microarray for onsite VOC monitoring.

Keywords: Cell-based sensor, VOCs, surface plasmon resonance, electrochemical impedance spectroscopy

Volatile organic compounds (VOCs) are important indoor air contaminants in industrial, commercial, and occupational environments. Exposure of animals to VOCs causes disease by diffusion of these compounds into epithelial cells [25]. Once VOCs have penetrated cells, they produce proteins that result in a certain level of host damage. It has been demonstrated that VOCs are capable of causing lipid peroxidation of human blood plasma. Even with low exposure levels, alterations in immune reactivity resulting in a subsequent higher risk for the development of an allergic response have been reported [13, 19]. The results obtained with traditional constant-flow exposure systems have been difficult to reproduce, and do not allow for the analysis of small-scale dose-response relationships of VOC effects. Thus, attempts to establish a simple *in vitro* exposure system that can be used to quantify oxidative stress changes on health effects caused by VOCs have been technically challenging.

Cell-based sensor arrays are potentially useful for the investigation of cytotoxic and immunomodulatory effects of VOCs. The arrays offer several advantages over traditional systems [6]. First, direct living cell immobilization is possible and allows for monitoring of simultaneous exposure to a series of doses. Second, sensing arrays can be measured in real time with high throughput. Third, electrical sensing devices can be used for signal-frequency patterns in cell growth medium; biocompatible materials and surface modifications are important processes in the fabrication of cell-based sensors [28]. Surface functionalization, based on extracellular matrix proteins that interact with cell adhesion molecules, may be a reliable candidate for cellular attachment onto specially designed surface patterns without loss of cell viability. The cell adhesion motif RGD (Arg-Gly-Asp) and its derivatives, found in a variety of cell adhesion molecules, have been used widely as substrates that support cell adhesion. A modified RGD peptide that terminates with a cysteine (Cys) amino acid can be self-assembled onto a gold (Au) sensing surface [21, 29]. Some investigations have supported the suggestion that the cell biocompatibility

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of materials is related to their surface electrochemical impedance [18]. The electrochemical impedance spectroscopy (EIS) measurement is an electrochemical method by which the material surface character can be analyzed without interfering with the surface electrochemical behavior [16]. However, the electrochemical character of the VOC-sensing Au surface, cultured with living cells, has not been actively evaluated.

In this study, a designed oligopeptide was utilized for cell immobilization; subsequently, a VOC detection system that enabled cell lines to be simultaneously exposed to defined concentrations of VOC was evaluated to determine dose-response relationships. The oligopeptide immobilized on the Au surface was anticipated to promote binding of the target cell; the chemical features of the oligopeptide may have influenced the binding capacity of the cell immobilization. Branched oligopeptides that are sequenced as C-R-G-D-R-G-D-R-G-D-R-G-D [C(RGD)$_4$] were applied for the formation of a cell immobilization platform. The formation of a self-assembled peptide layer on the Au substrate and the immobilization of HeLa (Human epithelial carcinoma cell line) cells were evaluated by surface plasmon resonance (SPR) spectroscopy. In addition, electrochemical impedance spectroscopy was studied on the immobilized HeLa cells with respect to VOC exposure. The results of this study will aid in the evaluation of VOC cytotoxicity by monitoring the time- and dose-response relationships of specific toxicological endpoints.

Materials and Methods

Materials

Benzene, toluene, xylene, and chlorobenzenes were obtained from the Aldrich Chemical Company. Branched oligopeptides C(RGD)$_4$ were purchased from Peptron (Korea). In addition, 11-mercaptoundecanoic acid (11-MUA) and phosphate, buffered saline (pH 7.4, 10 mM) were purchased from Sigma-Aldrich (U.S.A.). Other chemicals used in this study were obtained commercially as reagent grade compounds.

Cell Culture

HeLa cells, from a human epithelial carcinoma cell line, were obtained from the Korean Cell Line Bank (Korea). All cells were cultured in DMEM (Gibco, Rockville, MD, U.S.A.) supplemented with 5% heat-inactivated FBS (Fetal Bovine Serum) (Gibco), 0.37% sodium bicarbonate (Sigma, St. Louis, MO, U.S.A.), and streptomycin/penicillin (Gibco) at 37°C in the 5% CO_2 incubator.

Cell Toxicity Assay

A home-made culture chamber with Teflon (volume: 5 ml; culture surface: 4 cm^2) was used for the cell culture because the aromatic hydrocarbons interacted with the polystyrene walls of standard culture flasks [11]. After seeding (10^5 cells/chamber), cells were allowed to attach and the pH of the medium allowed to equilibrate for 12 h. Then, each VOC was introduced and the chambers were rapidly covered. Cell proliferation was estimated by counting cells in the suspension after trypsin-EDTA treatment, using a Coulter Counter. In all cases, three cultures were treated in parallel for each pollutant concentration and three control cultures were processed similarly for comparison.

Western Blotting Hybridization

The cell extracts were electrophoresed for 2 h with SDS-PAGE at 10 mA, and blotting was performed for 1 h and 30 min with a Hybond-ECL membrane (Amersham, Uppsala, Sweden) at 100 volts. The blotted membrane was blocked with 5% skim milk and reacted with primary antibody, mouse anti-human proteins (Novocastra, Newcastle, U.K.) and secondary antibody, horseradish peroxidase-conjugated goat anti-mouse IgG (Novocastra, Newcastle, U.K.). For each step, the membrane was washed with TBST (Tris-buffered saline with 0.1% Tween-20) three times. Protein bands were visualized using an ECL Kit according to the manufacturer's protocol (Amersham, Arlington Heights, IL, U.S.A.).

Fabrication of Oligopeptide Layers by the SA Technique

Gold substrate was prepared by DC magnetron sputtering on the cover glass (BK7, 18 mm×18 mm, Superior, Germany) and the designed glass (SF10, 18 mm×18 mm× 1 mm, Korea Electro Optics, Korea) for flow type SPR, respectively. Before the gold sputtering, chromium (Cr) was sputtered onto the glass to promote the adhesion of Au. The thicknesses of the Au and Cr films were 43 nm and 2 nm, respectively. Before the fabrication of the oligopeptide layer, the Au surface was cleaned using a piranha solution. A thin film of C(RGD)$_4$ was fabricated on the gold surface by submerging the substrate into the solutions (0.1 mg/ml of C(RGD)$_4$) for at least 24 h, as reported previously [9]. Finally, the prepared oligopeptide surfaces were washed with deionized distilled water and dried under N_2 gas.

SPR Spectroscopy

SPR spectroscopy was carried out according to the manufacturer's instructions (Multiskop, Optrel GbR, Germany) [9]. A home-made flow cell with the Teflon tubing and chamber was applied for VOC detection. The flow rate was controlled with a peristaltic pump (MP-3N, EYELA, Japan). The SPR measurement system was composed of a He-Ne laser used as a light source to make a monochromatic light with a wavelength of 632.8 nm, a polarizer, an analyzer, and a photo-multiplier tube (PMT). Regardless of the detection type, a 90° glass prism, similar to the substrate, was used for the configuration of the Kretschmann ATR coupler. The plane face of the 90° glass prism was coupled to a glass slide *via* index matching fluid (benzyl benzoate, Merck, Germany). The resolution of the angle reading of the goniometer was 0.001°.

Electrochemical Assay

Electrochemical investigations were carried out in a three-electrode cell configuration (CHI-660, CHI, U.S.A.) by means of cyclic voltammetry and impedance spectroscopy. Potentials were measured relative to an aqueous, saturated Ag/AgCl double junction (as reference electrode) and a platinum wire (auxiliary electrode). In addition, we used sine waves between the HeLa cell-immobilized electrode with an amplitude of 5 mV for frequencies in the range 10^{-3}–10^5 Hz. The experiments were run at an applied bias potential of 0.8 V of the $[Fe(CN)_6]^{3-/4-}$ redox couple, with 5 mV (rms) sinusoidal excitation amplitude. The impedance measurements were

carried out in the presence of DMEM as a redox probe at the formal potential of the system. The impedance spectra were plotted using complex plane diagrams [14]. Data acquisition was conducted using CHI-660 software for impedance measurement. Electrochemical experiments were conducted using a well-type minicell fitted with a cell-immobilized gold-coated electrode as the working electrode (0.64 cm^2). All measurements were carried out at ambient temperature.

Nitric Oxide Assay

NO was assayed by measuring the end-product nitrite, which was determined by a colorimeter assay based on the Griess reaction. Cell culture media were harvested after incubation of cells in the absence and presence of VOCs for 48 h at 37°C. The nitrite levels were determined using a modified Griess reagent (Sigma) following the manufacturer's manual. Briefly, the supernatant (0.5 ml) was mixed with 0.5 ml of Griess reagent at room temperature for 10 min. The OD of the mixture was measured at 540 nm after 15 min. The concentration of nitrite was determined by reference to a standard curve of sodium nitrite (Sigma).

RESULTS

Cell Growth Inhibition by VOCs

After incubation for 24 h, cytopathic effects (CPE) could be seen in the cell using converted microscopy. The cytotoxicity of each VOC, at 0–500 μmol, was determined on the HeLa cells (data not shown); at above 500 μmol, significant cytotoxicity was observed in the cells. To evaluate the different cytotoxic effects of each VOC on the growth of the HeLa cells, we added VOCs to the cell lines for four days at increasing concentrations. As shown in Fig. 1, exposure with increasing concentrations of VOCs resulted in inhibition of cell growth in a concentration-dependent manner. Cytotoxicity studies showed that xylene was much more harmful than any of the other VOCs.

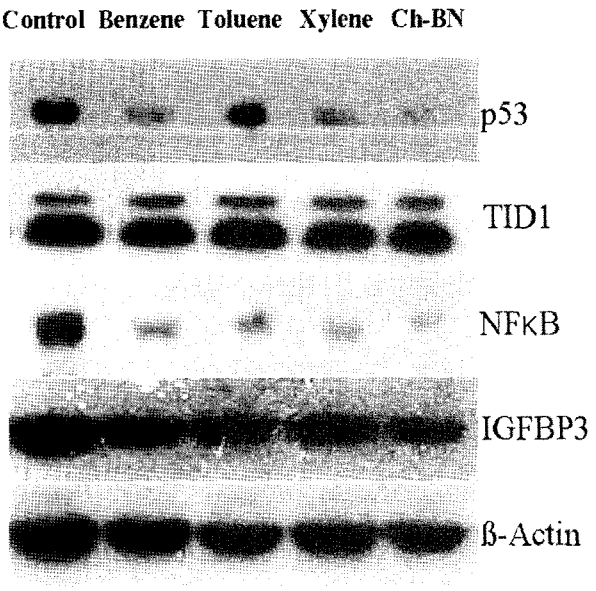

Fig. 2. Western blot analysis of stress-related proteins *in vitro*.
Total extracts obtained from the HeLa cells without VOCs treatment and with VOCs treatment were subjected to Western blot analysis, as described in Materials and methods.

By contrast, no significant changes in cell viability and proliferation were observed when cells were treated with PBS as a negative control; the findings suggested that the inhibition of cell growth was mediated solely by the exogenous VOCs.

To determine whether the VOC overexpresses stress-related proteins in the HeLa cells, Western blot analysis was performed after VOC exposure. As shown in Fig. 2, the results showed that p53 (transformation-related protein) had a low level of expression in HeLa cells on day two. Note that the same trends in NF-κB (transcription factor) and IGFBP3 (insulin-like growth factor-binding protein)

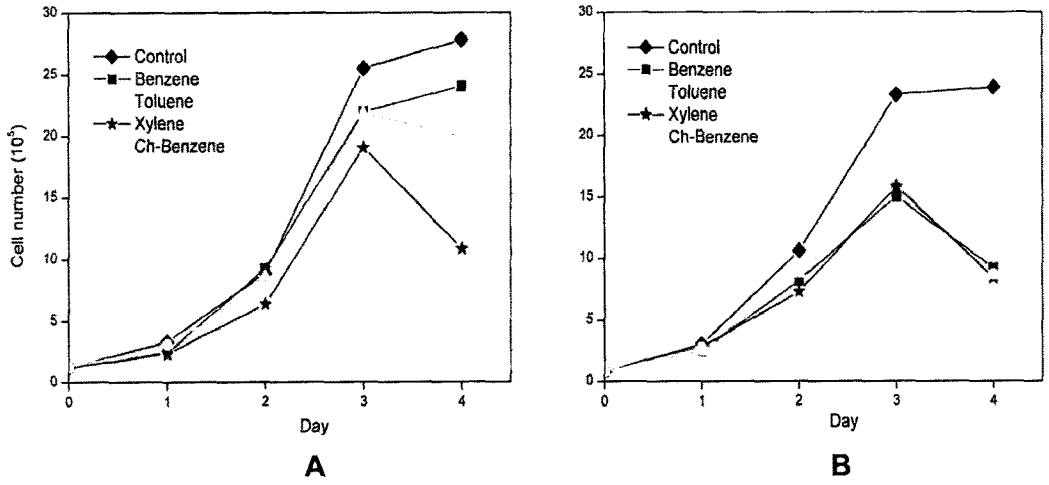

Fig. 1. Effects of VOCs on the growth suppression of HeLa cells.
Cells were treated with the indicated amount of VOCs, 500 μmol (**A**) and 1 mM (**B**), for 4 days of incubation. Cell growth suppression was measured as described in Methods and Materials. The assay was performed in triplicate.

Table 1. SPR angle shift showing binding of HeLa cells onto each surface.

	24 h	48 h
Bare Au	1.40°	1.26°
11-MUA	1.07°	1.12°
C(RGD)	1.17°	1.31°

expression levels were observed. There was no change in expression of TID1 (DnaJ homolog) in the VOC-treated cells. The results suggest that VOC was responsible for changes in stress-related protein expression in the HeLa cells.

Cell Immobilization

Three layer types including the bare gold layer, the 11-MUA layer, and the synthetic oligopeptide $C(RGD)_4$ layer, were prepared on the gold surface for the immobilization of the HeLa cells. When the HeLa cells were immobilized on each specific Au surface, the minimum position of plasmon angle was moved forward; this was caused by an increase in the amount of cells immobilized on the surface layer. As shown in Table 1, the minimum position of the plasmon angle shows the deposition of the HeLa cells on each surface for 48 h. On the basis of the bare Au substrate, the plasmon resonance angle was significantly increased during immobilization of the HeLa cells over a period of 24 h. In addition, the SPR angle shift of the bare gold surface with the HeLa cells was 1.4 to 1.26 over a period of 48 h. After the self-assembly formation of the 11-MUA layer onto the bare Au substrates, the HeLa cells were introduced onto the surface. When the cell immobilization process was evaluated with SPR, the subsequent binding of the HeLa cells led to a low shift in the angle of the plasmon resonance over 48 h. However, after the self-assembly formation of the $C(RGD)_4$ layer onto the bare Au substrates, the subsequent immobilization of HeLa cells on the $C(RGD)_4$ oligopeptide layer resulted in a SPR angle shift to 1.31° over 48 h. Therefore, the self-assembled synthetic oligopeptide layer was fabricated on the bare Au substrate and the subsequent HeLa cells were more actively immobilized on the oligopeptide layer.

Electrochemical Detection

For the application to the cell-based VOC assay, we measured the electrochemical signal changes. The measurements were based on Faradic impedance in the presence of the redox couple of hexacyanoferrates. A minimum concentration of ferro/ferricyanide was applied to the cell culture system because the effects of subjecting the cells to ferro/ferricyanide might have stimulated a response in the cells. There were distinctive characteristics observed in the normal cell electrodes compared with the VOC-treated electrodes. Fig. 3 shows the cyclic voltammograms of $[Fe(CN)_6]^{3-/4-}$ recorded

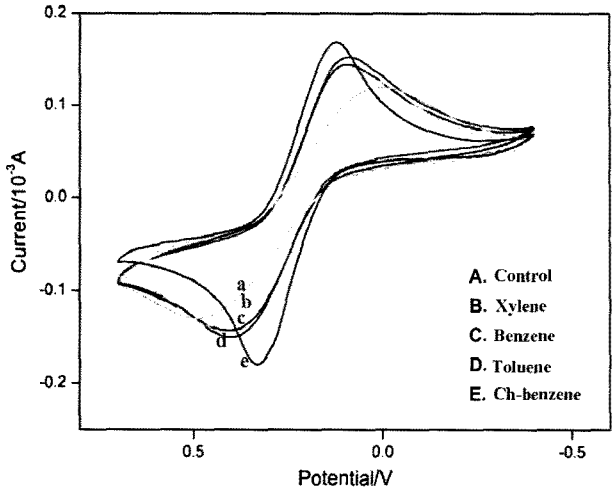

Fig. 3. Cyclic voltammograms of a cell-based sensor with (**A**) control and (**B–E**) VOCs exposure in 0.1 M KCl containing 2 mM $[Fe(CN)_6]^{3-/4-}$ (1:1 mixture); scan rate, 0.1 V/s.

for the Au electrodes modified with the HeLa cells (curve A) and with the VOC exposure (B–E). As expected, different signal patterns for the VOCs were identified in the HeLa cells. The quality of the voltammograms improved as the sensor was exposed to a certain toxin. Impedance spectra showed a high current for the VOC exposure compared with the normal HeLa cells. Each VOC, however, did not induce a VOC-specific change in the spectra analyzed. It was noted that the cells were not dislodging from the electrode over time; in addition, the VOCs were not removing the peptide monolayer and not affecting the active area of the electrode and therefore its electron transfer properties (data not shown). Fig. 4 shows the impedance spectra obtained in 0.1 M KCL with $[Fe(CN)_6]^{3-/4-}$

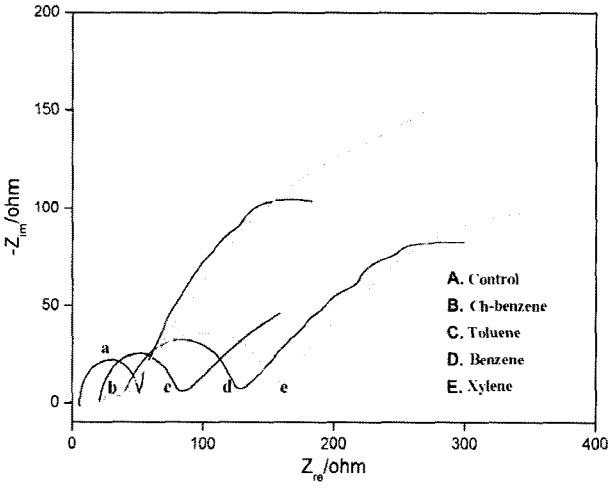

Fig. 4. Nyquist plot (Z_{im} *vs* Z_{re}) for the Faradaic impedance measurements in 0.1M KCl containing 2 mM $[Fe(CN)_6]^{3-/4-}$ (1:1 mixture).
The experimental data points are shown as (**A**) control and (**B–E**) VOCs exposure.

when treated with VOCs. The results showed the effect of the HeLa cell-VOC interaction on the Nyquist plot as the sum of the real and imaginary components (Z' and Z''). Each VOC caused a change in the spectra, which was due to a change in the electrolyte properties adjacent to the cell-based electrodes after the exposure. Note that the VOC-specific trend in electrochemical signal levels could be identified in the HeLa cells. The spectra showed lower impedance for the normal HeLa cells than for cells after VOC exposure. There were large increases observed during highly toxic xylene exposure and less of a change noted after moderate toxic chlorobenzene exposure. These measurements showed that the impedance was very stable and reproducible over the time frame of the measurements and over the entire frequency range. The Nyquist plot more clearly emphasized the impedance changes at high frequency, where the experiments showed the greatest sensitivity to the presence of the VOCs. Exposure of the HeLa cells to the VOCs resulted in increased impedance and a semicircle line. The results provide evidence for the existence of a VOC-dependent infusion into the HeLa cells. Therefore, the EIS can detect the specific presence of VOCs more clearly than CV measurements.

NO Detection

Because NO seems to play a key role in regulating animal defense responses, we examined the potential of the VOCs to induce an increase in NO in the HeLa cells. To quantitate NO in the VOC-treated HeLa cells, a spectrofluorometric assay was developed; the end-product nitrite was measured, which was determined by a colorimeter assay based on the Griess reaction. Fig. 5 shows the real-time monitoring of NO production in the HeLa cells after VOC treatment and subsequent cell culture. As shown, VOCs were observed to be strong inducers of NO production. In addition, the VOC treatment resulted in an increase of NO concentration, indicative of a cell defense response. The NO production was apparent even at 24 h after VOCs exposure. Furthermore, cytotoxicity was observed, using a converted microscopy, up to 48 h after VOC exposure, even though the levels of NO remained unaffected. Note that the NO production trends showed an increased cell response in a manner similar to the electrochemical impedance trends shown in Fig. 4. By contrast, there was no correlation with the cyclic voltammograms, as shown in Fig. 3. Therefore, the EIS can detect more clearly changes of constitution of electrolytes influenced by the cell immune response compared with CV measurements.

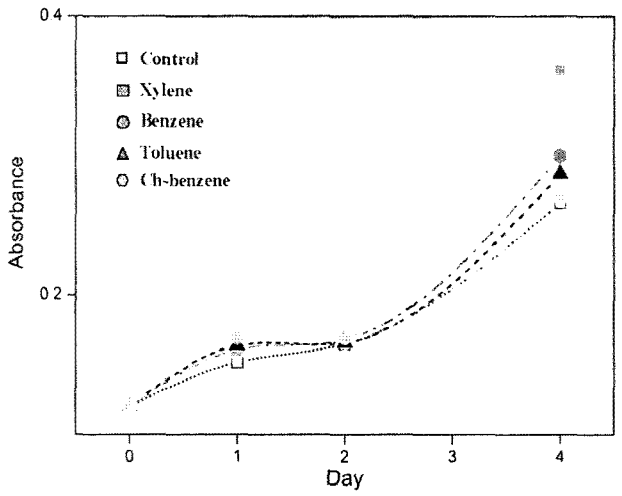

Fig. 5. Time-course of NO changes after VOCs exposure. NO production was estimated by Griess reaction with a microplate reader.
The values represent a mean of three independent experiments.

DISCUSSION

It is known that when immobilized cells are exposed to VOCs, cell viability changes and hydrogen peroxide production varies in accordance with cell damage and metabolic activity [8, 12]. These results have been confirmed by the suppressive influences on cellular viability and defense mechanisms by increasing cell death during oxidative stress [5, 33]. In this study, we introduced a new cell-based assay system that employs electrochemical measurement to determine specific VOCs. The viability of HeLa cells, immobilized on a branched $C(RGD)_4$ network, was studied to detect the biological toxicity of VOCs. Viability of the cell cultures exposed to VOCs, with the cell-based system used in this study, proved to be dose-dependent and highly sensitive.

Surface plasmon resonance characteristics were investigated to monitor surface modification and cell immobilization. When the sensor surface is coated with a single sensing cell, the method can be used for an affinity binding study and the detection of a selected drug in a sample mixture [30]. A SPR sensorgram records the shift of a resonant wavelength as a function of time, and it can be used to quantify the amount of captured analyte. The amount of immobilized HeLa cells is important for electrochemical detection, because it can influence the sensitivity and reproducibility of the signal acquired by EIS and CV. The change of the plasmon angle with respect to the concentration of synthetic oligopeptides has been reported previously [18]. In this study, we immobilized a natural ligand, $C(RGD)_4$, for mammalian cell adherence onto the sensor chip surface, and demonstrated that the surface was stable over a period of several consecutive days. A control surface without $C(RGD)_4$ showed nonspecific binding to the experimental surface. The HeLa cells strongly adhered to the bare Au for 24 h. Furthermore, morphological changes, such as extended pseudopods and flattening, were observed in the adhered cells. The reduction of cell adhesion

over 48 h was related to cell viability and conformational changes on the hydrophobic Au surface. The 11-MUA layer was prepared on hydrophobic gold surfaces, which helped to decrease the cell adhesion *in vitro*. Therefore, the monolayer did not offer enough stability for the physically adsorbed HeLa cells; this finding suggests that the 11-MUA monolayer has a non-adhesive property for cells [26, 31]. This specific character of the monolayer is explained by the dynamic motion of flexible chains and the hydrophilic nature of the terminal carboxylic acid [23]. Therefore, a bulky and extended biocompatible layer was needed to effectively enhance cell adhesion, as shown in the case of $C(RGD)_4$.

For the application to a cell-based VOC assay, we used the immobilized HeLa cell substrate for exposure to the VOCs, and measured the electrochemical impedance changes for VOC detection. The results showed the effect of the HeLa cell-VOC interaction on the Nyquist plot as the sum of the real and imaginary components (Z' and Z") [20]. The plots clearly show changes in impedance based on the VOC-specific exposure. The plot more clearly emphasized changes in impedance at high frequency, where the experiments showed the greatest sensitivity to the specific presence of VOCs. In addition, the VOC treatment of the HeLa cells resulted in increased impedance and a straight line in the low frequency ranges. Thus, the Nyquist plot for the HeLa cell-immobilized gold substrate can be described as a semicircle near the origin at high frequencies, followed by a linear tail with a slope of unity. Because the toxicity of VOCs is very high, the cell response is expected to be irreversible. It is clear that the cell response (inflammatory response) dramatically causes changes in electrolytes close to the electrodes. EIS spectra have provided a great deal of information, including solution resistance, electron transfer resistance, interfacial capacitance, and mass transport. In this study, there was comparatively greater influence of the cell response on the charge dispersion at the electrode double layer. The interface boundary is ascribed to the electrode in contact with the electrolyte, and the contact could be in the form of an extremely thin aqueous film [27]. It has been shown previously that the double layer of cell-based surfaces is characterized by a dispersive capacitance. Thus, the results indicated that the changes in the impedance signal were from the VOC toxicity.

We used four different VOCs from an array of animal-associated toxins. Here, we demonstrated that VOCs such as xylene, benzene, toluene, and chlorobenzene induced immediate production of NO in the HeLa cells. The development of sensitive and selective analytical techniques for the measurement of NO in biological systems has been a matter of great interest [3, 34]. NO was assayed by measuring the end-product nitrite in concentrations as low as ~0.5 mM; determined by a colorimeter assay based on the Griess reaction that has been used extensively in analysis of numerous biological samples including plasma (serum), urine, CSF, saliva, and cell culture media [2]. In this study, we demonstrated that xylene was much more cytotoxic than any other VOCs. Exposure to VOCs has been shown to induce synthesis of cytochrome P450 and aldo-keto reductase, which lead to the formation of the reactive oxygen species (ROS) [7]. In addition, it has been reported that the cytotoxicity of VOCs is related to their lipophilicity, increasing in order of benzene<toluene <xylene [32], which correlates well with our NO assay results. Investigations of the mechanism(s) by which the VOCs affect the immune reaction have been the subject of many studies. In the HeLa cells, NO-mediated signal transduction is sensitive to VOCs in the low molecular weight range. NO-derived reactive species such as peroxynitrite are known to be responsible for the cytotoxicity and tissue injury associated with increased NO production [1]. In this study, the HeLa cells responded strongly to the reduction of stress-related proteins. The p53 and NF-κB expression levels in the HeLa cells were significantly down regulated by VOC exposure. However, it has been reported that *in vitro* exposure caused significant increases of TNF-α secretion and NO generation in a time-dependent manner, suggesting the progressive development of an inflammatory response [4]. In addition to their oxidative effects, reactive oxygen species (ROI) have been implicated in the regulation of NF-κB, and therefore of TNF-α. HSP72 and HSP90 levels in HepG2 cells and normal fibroblasts were not significantly upregulated after exposure to VOCs [10]. In addition, higher toluene concentrations suppress cytokine secretion as well as the activity of lymphocytes, whereas the production of pro-inflammatory cytokines (TNF-α) has been reported to be induced [15, 22, 35]. Thus, in this study, it is likely that inactivation of p53 and NF-κB was related to VOC-specific oxidative stress, leading to increased levels of abnormal proteins in the HeLa cells.

NO can be produced by NOS-like enzymes or by nitrate reductase (NR) [24]. To determine which VOC significantly activates the NO-source, we first reported a molecular biological approach. Xylene-induced NO production in the HeLa cells was dramatically increased. However, the chlorobenzene-induced NO increase was insensitive, indicating that a VOC-specific cell response was involved in the NO synthesis. The presented data clearly demonstrate that significant NO production, after VOC exposure, showed increased cell response in a manner similar to the electrochemical impedance trends. Further study is needed to explain the effects on the electrolyte solution. In this study, we could confirm that the production of NO around the cell-based electrode surface followed VOC exposure and significantly influenced the impedance, in particular the impedance in the high frequency region. The findings

from this study suggest that the increase in impedance was due to the changes in electrolytes close to the electrode resulting from the cell inflammatory response [17]. The difference in the impedance of the normal cell electrodes and the VOC-treated electrodes demonstrated the influence of the presence of a biofilm on the electrode surface when aqueous electrolytes were changed by cell responses. Since electrode impedance depends on electrode surface area and composition of the electrolytes, it can be very useful to study the electrode interface on immobilized living biological environments and its effect on impedance.

Based on this concept, the changes of electrochemical impedance spectroscopy with respect to VOC-specific toxicity have been illustrated. From these results, the detection of biological toxicity can be successfully carried out based on immobilized cells and EIS. In addition, the designed synthetic peptides could be applied to the fabrication of a biocompatible surface for cell immobilization. The strong response of the HeLa cells to the VOCs suggests that the cell-based assay system can be used for VOC-specific detection of electrochemical signals.

In conclusion, the present study demonstrated the feasibility of a VOC-specific detection system in mammalian cells immobilized on a biosurface composed of a synthetic oligopeptide that can be applied on a cell chip platform. In addition, the results establish the utility of impedance spectroscopy for a direct electrochemical detection technique to monitor VOC-specific exposures. Because the damage following VOC exposure can have a significant impact on cell viability in the immobilized HeLa cells, EIS was successfully observed with respect to the cell damage after VOC-specific exposure. The proposed cell immobilization method using a self-assembly technique can be applied to construct a cell chip for onsite VOC monitoring.

Acknowledgments

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