Anal. Chem. **2008,** *80,* 990−996

Real-Time Monitoring Primary Cardiomyocyte Adhesion Based on Electrochemical Impedance Spectroscopy and Electrical Cell−Substrate Impedance Sensing

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The cell−substrate distance is a direct indicator of cell adhesion to extracellular matrix which is indispensable in cell culture. A real-time monitoring approach can provide a detailed profile of cell adhesion, so that enables the detecting of adhesion-related cell behavior. In this work, we report a novel real-time impedance-based method to record the adhesion profile of cardiomyocyte, overcoming its inscrutability due to the primary culture. Microfabricated biosensors are applied in cardiomyocyte culture after characterizing the cell-free system. Cyclic frequency scanning data of cell-related impedance are generated and automatically fit into the equivalent circuit model, which is established using electrochemical impedance spectroscopy. The data are displayed as the alteration of normalized cell−substrate distance and the essential parameters for manual electric cell−substrate impedance sensing calibration of absolute distance. The time course displays a significant decline in the equivalent cell−substrate distance, from 155.8 to 60.2 nm in the first 20 h of cardiomyocyte culture. Furthermore, the cardiomyocytes cultured in long-term medium and short-term medium (ACCT) for 10 h exhibit distinct difference in adhesion rate as well as cell−substrate distance (72 vs 68 nm).

The ability to culture primary cardiomyocytes has resulted in the availability of a well-characterized in vitro system in which adhesion of cardiomyocytes to the extracellular matrix (ECM) is an important behavior. Research has proven that adhesion builds up the ECM-dependent signaling pathways for the cardiac hypertrophy,[1] apoptosis,[2] and migration, among other processes. Inside the cardiomyocytes, which are undergoing specific pathways, the formation or elimination of cell focal adhesions occurs as side tracks of signaling pathways. This leads to the strengthening or weakening of cell adhesion. In other words, the alteration in cell adhesion is a cellular level response of cardiomyocytes to certain processes, which enables real-time monitoring of the cell adhesion to provide instant information regarding cell behavior. In particular, compared with qualitative optical observation, the acquisition of the change rate of cardiomyocyte adhesion will be a significant advancement in the field. Therefore, the need exists to develop a new biosensing system for real-time monitoring of cell adhesion, which is quantitative, convenient and noninvasive.

It has long been recognized that electric cell−substrate impedance sensing (ECIS) is valid and holds promise for monitoring the morphology, viability, and environmental change of the adherent cells.[3,4] ECIS is a noninvasive and sensitive detecting technique for cell adhesion. In the last two decades, ECIS sensing has been employed to monitor many types of cells, including fibroblast, endothelial cells, etc.[5−11] More recently, ECIS research turned to excitable cells, such as skeletal muscle cells[12] and cardiomyocytes.[13,14] Generally, time courses of overall impedance during the biological processes were recorded in time intervals and attributed to various cell behaviors.

Despite the impressive advances in ECIS research with time courses of overall impedance, current and future studies strive to provide direct information of ECIS through advanced analyses. Giaever developed the data processing techniques catered to his ECIS results. In his method, paracellular current flow in the medium gap between disklike cells and electrodes is described

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10.1021/ac701745c CCC: $40.75
Published on Web 01/24/2008

in the infinite element model,[15] where the cell body is assumed exhibiting known and stable capacitance. It has been reported that this advanced ECIS method is capable of detecting vertical motion in the order of 1 nm[4]. Lo[16] further developed an advanced ECIS method with the cell model constructed with a flat rectangular box and a half disk on each end, enabling the advanced ECIS to cells that are not so symmetric as that of Giaever's. However, unlike endothelial cells or fibroblasts in the reported ECIS cases that show known and constant electrical properties due to the reliable commercialized cell lines, cardiomyocytes are primarily cultured, which means membrane impedance of cardiomyocytes are unknown and various. Furthermore, Lo's model has two adjustable parameters, which are to be determined in the curve fitting. The fitting methodology is developed from a nonlinear optimization in which an initial guess is one of most important criteria. The iterative process can converge too slowly or even fail without a reasonable initial guess. Unfortunately, these adjustable parameters are absent in the research on cardiomyocytes whereas those of endothelial cells and fibroblasts are reported and then utilized in the ECIS for these types of cells. These factors are essentially the bottleneck of ECIS application to cardiomyocyte research.

In this research, electrochemical impedance spectroscopy (EIS), a powerful approach to the study of electrolyte−electrode interface, is invoked to the impedance sensing and analysis. Frequency scanning is applied in electrical measurements instead of recording impedance data at a single frequency. Impedance data are used to determine the components of equivalent circuit models based on EIS. We show that, as cardiomyocytes are cultured on the biosensors, the resistance of the cell−substrate gap exhibits the increscent tendency. This process can be manually calibrated with ECIS and translated into a decreasing time course of an equivalent cell−substrate gap. Moreover, the comparison of cardiomyocytes cultured in long-term medium (LTM) and ACCT demonstrates the distinction of cell adhesion in different media. These results support cardiomyocyte adhesion processes observed in biological experiments and validate our impedance sensing and analysis system for real-time monitoring of cardiomyocytes.

EXPERIMENTAL SECTION

Fabrication. The fabrication of the biosensing chips was conducted in a microfabrication laboratory. A glass slide was chosen as the substrate material because it could minimize the substrate capacitance and decrease the measurement noise. The electrode voids of the chips were patterned on the slides with photoresist Shipley 1813 in photolithography on the MA6 aligner. Effective adhesion of the metal electrodes to the glass substrate is required; therefore, a chromium layer (125 Å) was first deposited in thermal evaporation, followed by the deposition of the major electrode components with gold (375 Å) (Kurt J. Lesker Co.). The electrodes were subsequently defined in a liftoff process. SiO_2 and SiN_x (Kurt J. Lesker Co.) were deposited with magnetic sputtering to form an insulating layer 1200 Å thick. The metal electrodes were exposed in reactive ion etching process. Figure 1 shows the configuration of the biosensing chip.

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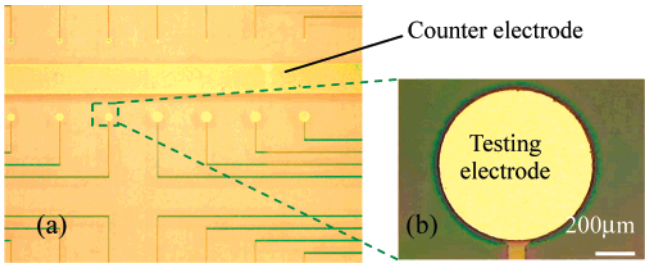

Figure 1. (a) Top view of the bioimpedance sensor. (b) Close view of the testing electrode fabricated with liftoff methods (Cr/Au: 125 Å/375 Å). Chromium was used to increase the adhesion of the gold layer to the glass substrate. A laminin (an ECM protein) layer was coated before cardiomyocytes were injected.

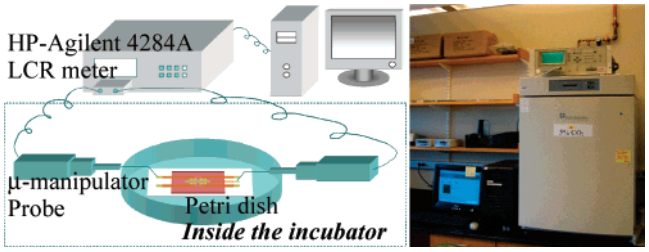

Figure 2. Schematic diagram and photo of the impedance measurement system. The part surrounded by dash lines is inside the incubator (37 °C, 5% CO_2).

Testing Systems. The schematic diagram and photo of our impedance-sensing system is depicted in Figure 2. The biosensing chip was maintained in the incubator at 5% CO_2 and 37 °C throughout the electrical measurement to avoid any fluctuations in the testing environment. Inside the incubator, the biosensing chip was mounted to a homemade silicone chamber designed for cell culture. The electrode pads were connected to micromanipulators, which transferred signals through an Agilent 16048A BNC test fixture to the outside of the chamber. The impedance of the electrodes in the chip was measured with an Agilent 4284A LCR meter (Agilent Technologies Inc.). For automatic measurement and data logging, the LCR meter was connected to a computer through a GPIB interface. The impedance measurement process was controlled by LabView (National Instruments Corp., Austin, TX) virtual instruments.

Cardiomyocyte Isolation. Left ventricular myocytes were isolated from male Wistar rats according to a previously established protocol.[17] The left ventricle was separated, minced, and gently agitated allowing the myocytes to disperse in KB solution (85 mmol/L KOH, 30 mmol/L KCl, 30 mmol/L KH_2PO_4, 3 mmol/L $MgSO_4$, 0.5 mmol/L EGTA, 10 mmol/L HEPES, 50 mmol/L L-glutamic acid, 20 mmol/L taurine, 10 mmol/L 2,3-butanediaonemonoxime, and 10 mmol/L glucose). The cardiomyocyte suspension was subsequently filtered through a nylon mesh, resulting in a typical yield of >90% rod-shaped cells. The isolated cells were then washed with DMEM, with the addition of ascorbic acid (100 μM), and were ready for plating onto microelectrodes. The initial cardiomyocyte adhesion monitoring was performed to the cells in DMEM for the first 20 h following the initiation of the culture.

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Electrical Measurement of Cardiomyocyte Adhesion. A laminin (Becton-Dickinson), the ECM protein, ACCT medium suspension was injected onto the testing electrode and maintained in a still incubator for at least 30 min until a laminin layer formed. The characterization of the laminin layer formed on the testing electrode was then performed with 80 frequencies logarithmically spaced between 20 Hz and 200 kHz, with a 5-mV voltage excitation. The frequency scanning was completed in 1 min. After characterization, the laminin suspension was removed, and the cardiomyocyte suspension was injected in the silicone chamber. The electrical monitoring of cell adhesion was initiated immediately after the injection. The cyclic frequency scanning was applied to the testing system every 10 min in a 20-h cell culture period. The impedance data were recorded as real and imaginary components accompanied by the testing frequency in a txt file, which was convenient for the following data processing and analysis.

Cardiomyocyte Culture in Short-Term Medium and Long-Term Medium. Two different types of media were tested in this research, including (1) short-term medium (ACCT) (0.2 wt % albumin, 2 mM L-carnitine, 5 mM creatine, 5 mM taurine in low glucose DMEM); and (2) long-term medium (2 mM L-carnitine, 5 mM creatine, 5 mM taurine, 100 μM BrDU, and 7 vol % heat-inactivated fetal bovine serum in low glucose DMEM). After 1-h initial culture in DMEM, a comparison of cardiomyocyte adhesion in different media was performed using cardiomyocyte culture samples. The cyclic frequency scanning protocol was applied to the testing system in the following 10 h of cell culture. The same settings used in monitoring the initial cardiomyocytes adhesion were replicated.

RESULTS AND DISCUSSION

In this work, the time course of cell−substrate distance was extracted from the impedance data processed with the EIS and ECIS, which could be direct monitoring of cardiomyocyte adhesion to ECM. Equivalent circuit models for protein-coating and cell layers were built and modified according to the characteristics of impedance spectra. In this way, the mode of alternating current flow could be precisely described, which could further reveal the nature of cardiomyocyte adhesion. Of all elements in the equivalent circuit model, the resistance of the medium layer in cell−substrate gap was the most straightforward quantitative indicators describing the extent of adhesion. The dynamics of the adhesion process could be recorded by connecting the parameters of equivalent circuit determined in the cyclic frequency scanning. With manual ECIS calibration, the time course of equivalent cell−substrate distance was calculated for the cardiomyocytes under the following conditions: (1) the initial culture in DMEM; (2) the culture after the medium changed to ACCT; and (3) the culture after the medium change to LTM.

Characterization of Protein-Coated Gold Electrodes. Protein coating to the microelectrode surface is necessary for cardiomyocyte culture. However, the protein coating layer will cause an extra addition to impedance by blocking the excitation signal from the gold electrodes. It is necessary to separate the impedance components induced by the protein coating layer.

The impedance-sensing system without cells can be represented by the equivalent circuit model shown in Figure 3a. Here, the dielectric characteristics of the protein layer (laminin) are considered an ideal capacitor (C_p) on the electrode surface.

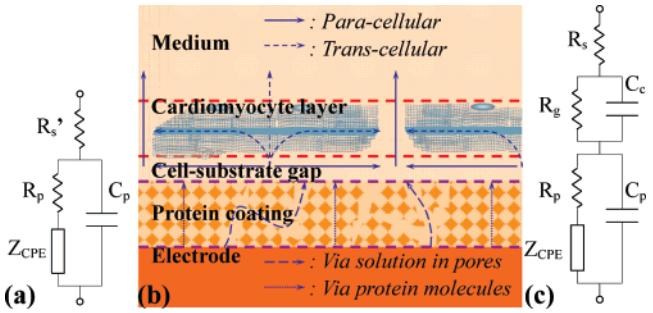

Figure 3. (a) Equivalent circuit model of system impedance without cells. R_s is the resistance of bulk solution and the wire connection. The capacitance of laminin layer is represented by C_p. Z_{CPE} and R_p are used to describe nonideal capacitive response of the uncovered electrode surface and the resistance in the pores of laminin layer. (b) Current flow patterns on the testing electrode. There are two modes of current in protein coating layer: one travels via solution in the pores among protein molecules; the other travels via protein molecules. There are also two modes of current in cardiomyocyte layer: Paracellular mode represents how the current travels in solution around cells; transcellular mode represents how the current travels through the cell body. (c) Simplified equivalent circuit model of impedance of the cell-covered electrode (Z_c). The impedance response to paracellular or transcellular current is described with resistors or capacitors respectively. Especially, R_g is the resistance of medium layer in the cell−substrate gap. When cardiomyocytes begin to adhere to ECM protein, the descent of the cell−substrate gap will result in the increase of R_g.

However, since laminin are macromolecules, which are ~900 kDa in molecular mass and ~10 nm in length,[18] it is difficult to achieve the complete and compact coverage of laminin over the electrode surface. Research does demonstrate the pores exist among the protein macromolecules.[19] Solution can enter these poles and reach the gold electrode surface (Figure 3b), which can induce nonideal capacitance and resistance. Therefore, a constant-phase element (CPE) is used to account for the nonlinearities of frequency-related electrical double layer impedance ($Z_{CPE} = [Q_{CPE}(j\omega)^n]^{-1}$, where j and ω is the imaginary unit and angular frequency, respectively) on the naked gold surface.[20,21] The resistor R_p is used to represent the pore resistance in the laminin layer. Both the current flows via protein molecules and via pores in protein coating layer travel in the bulk medium represented with a resistor (R'_s).

The equivalent circuit model was fit to the 3D impedance response plot of the impedance-sensing system, as shown in Figure 4. To determine the element parameters of the impedance-sensing system, a complex nonlinear least-squares (CNLS) curve fitting was performed to eq 1

$$Z_{nt} = R'_s + \frac{1}{j\omega C_p + \dfrac{1}{R_p + \dfrac{1}{Q_{CPE}(j\omega)^n}}} \qquad (1)$$

derived from the equivalent circuit model in Figure 3a: where Z_{nt} is the theoretical value of specific impedance of cell-free system (Z_n). The P weighting[22] was added into the objective function of

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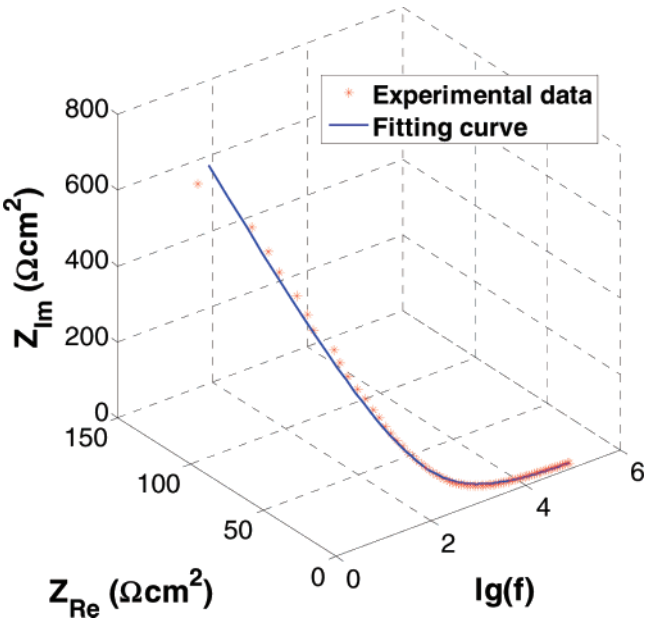

Figure 4. Characterization of cell-free impedance sensing system (Z_n) with CNLS curve fitting to eq 1 with the P weighting in the objective function. The curve fitting was performed with a program developed from optimizing function toolbox of MATLAB. According to the convention of the electrochemical society, $Z = Z_{Re} - jZ_{Im}$.

CNLS curve fitting (S) to ensure the validity in case the fitting to the real or imaginary component of impedance is unbalanced:

$$S = \sum_{i=1}^{k}\left\{\left[1 - \frac{Z_{nt,Re}(\omega_k)}{Z_{n,k,Re}}\right]^2 + \left[1 - \frac{Z_{nt,Im}(\omega_k)}{Z_{n,k,Im}}\right]^2\right\} \quad (2)$$

Despite the existing curve-fitting software, for example, LEVM, a CNLS curve-fitting program was written based on the optimizing toolbox of MATLAB. This allowed for a convenient means for the impedance data obtained in LabView virtual instruments and the following calculations and plotting. The element parameters are simultaneously determined: $R'_s = 4.39 \pm 0.05\ \Omega\cdot cm^2$, $C_p = 3.03 \pm 0.12\ \mu F/cm^2$, $R_p = 13.98 \pm 0.31\ \Omega\cdot cm^2$, $Q_{CPE} = 16.42 \pm 0.33\ \mu F/cm^2$, and $n = 0.827 \pm 0.003$.

Monitoring the Initial Adhesion of Cardiomyocyte. The monitoring technique of cardiomyocyte adhesion was developed based on the cyclic frequency scanning of impedance and calculation of cell−substrate distance, which began with separating the cell-related impedance (Z_r) from the overall (Z_c). According to the experiment protocol, the DMEM-containing laminin was drained and replaced with cardiomyocyte-suspended DMEM. Thus, the total impedance can be considered as the combination of Z_r and Z_n with the exception for R'_s. Therefore, Z_r values could be calculated ($Z_r = Z_c - (Z_n - R'_s)$).

To acquire more electrical parameters from the impedance measurement, we build the cardiomyocyte layer model on an electrode based on EIS, yet most assumptions in ECIS are

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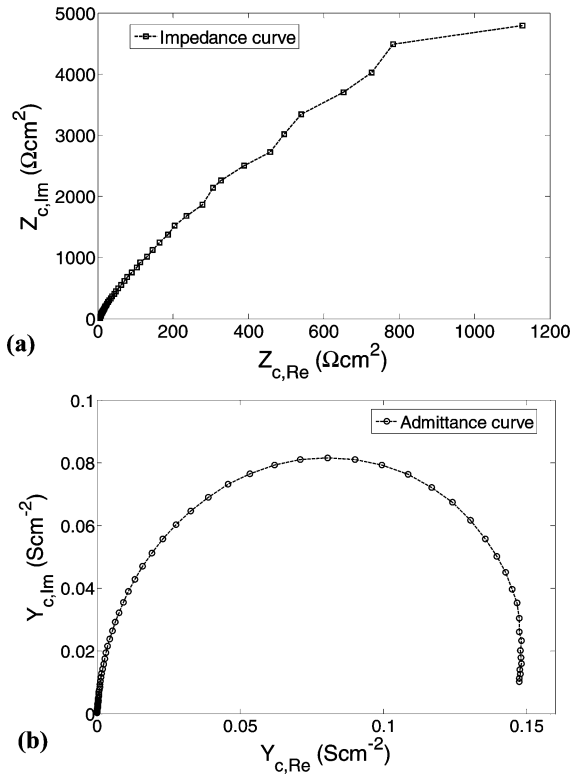

Figure 5. Typical Nyquist plots of Z_r and Y_r. These plots were taken automatically in the cyclic frequency scanning. The Nyquist plots of impedance and admittance are close to 1/6 of a circle and semicircle, respectively. Since the geometry of the admittance curve is more ideal than that of the impedance curve, it is easier to obtain the initial guess from the admittance curve than from the impedance curve.

maintained for the coherence of these two methods. As the testing voltage is applied to cells cultured on electrodes, the ac current flow is divided into the transcellular component and paracellular component, which travels via cells and via cell−substrate gap, respectively. Thus, in our equivalent circuit model of Z_r (Figure 3c), C_c is used to represent the reactance of cells and R_g is used to describe the resistance of the thin medium layer in the cell−substrate gap. R_s is the resistance of bulk medium body. Theoretical equation of Z_r derived from this model is

$$Z_{rt} = R_s + \frac{1}{j\omega C_c + (1/R_g)} \quad (3)$$

where Z_{rt} is the theoretical value of Z_r. The CNLS curve fitting with P weighting was employed here to determine the component values.

To achieve the automation in series of CNLS curve fitting, its initial guess is given by analyzing the characteristics of the impedance response in a Nyquist plot. When reversed, the impedance response data can be converted to admittance response ($Y_r = Z_r^{-1}$). The Nyquist plot of admittance response is a semicircle in the first quadrant that can be expressed as

$$(Y_{r,Re} - d - r)^2 + {Y_{r,Im}}^2 = (r/2)^2 \quad (4)$$

where d and r are the intercept and radius of the admittance semicircle. The electrical parameters, such as R_s and R_g, can be

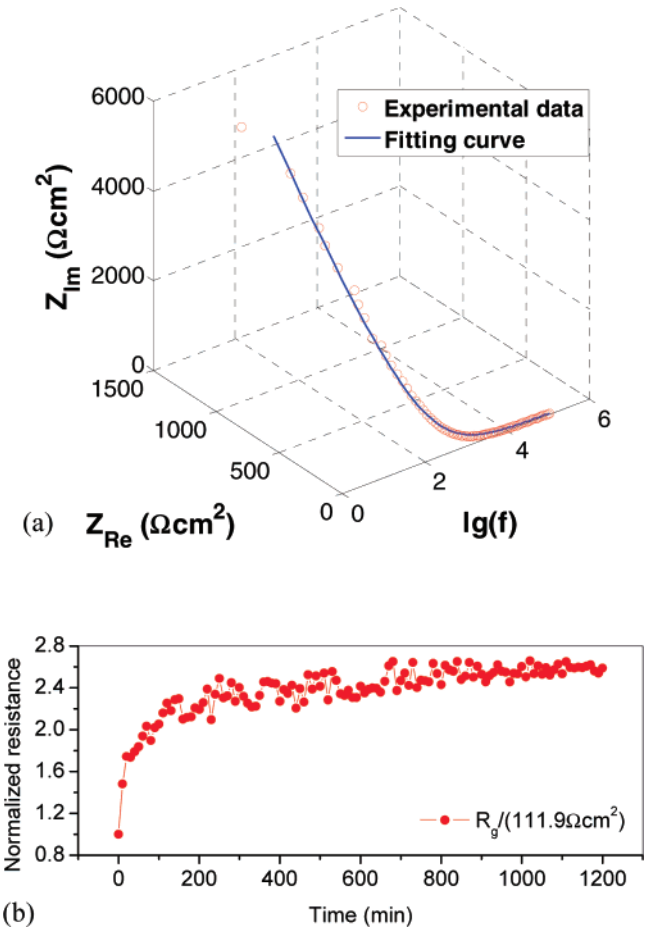

Figure 6. (a) CNLS curve fitting of the cell-related impedance to the EIS model (eq 3) with the *P* weighting in the objective function (eq 4). The related electrical parameters such as R_g and C_c are determined. (b) Alteration of normalized R_g in the first 20 h of cardiomyocyte culture. The initial value (R_{g0} = 111.9 Ω·cm^2) is set as normalizing units. R_g rapidly increased within the first hour and slowly reached its saturation within 20 h.

obtained by calculating eq 5 and eq 6, of which d and r are major parameters:

$$R_s = \frac{-d - r + \sqrt{9d^2 + 18dr + r^2}}{2d^2 + 4dr} \quad (5)$$

$$R_g = \frac{(r + d)R_s^2 - R_s^2}{2 - (r + d)R_s^2} \quad (6)$$

And C_c can also be calculated through eq 3. The reason for using admittance response instead of impedance response is the Nyquist plot of admittance is more orbicular than that of impedance, which is close to 1/6 of the circle (Figure 5).

The electrical parameters acquired in the Nyquist plots of Y_r are utilized as the initial guess of the following CNLS curve fitting (Figure 6a). Every loop in the cyclic frequency scanning goes through the EIS data processing to determine R_g, R_s, and C_c values.

The normalized time course of equivalent cell−substrate distance is defined based on normalizing the time course of R_g to its initial value. Its rationality is ensured by the following discussion. In our model, R_g describes the resistance to the

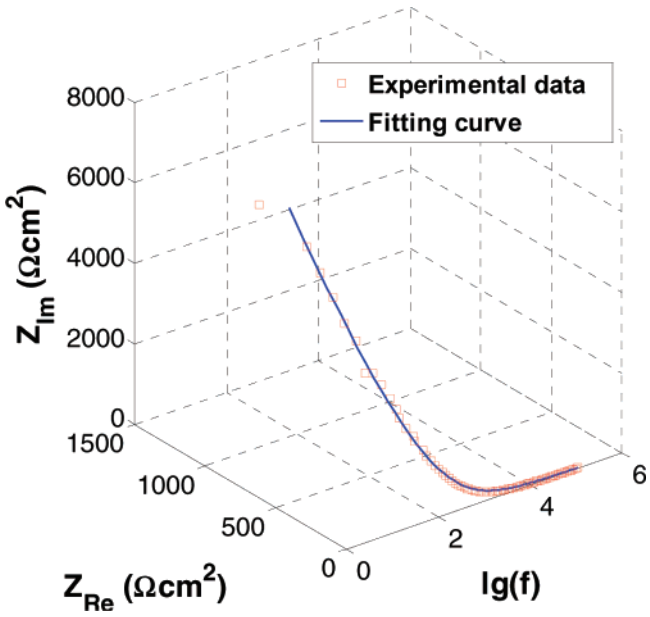

Figure 7. Calibration of the initial equivalent cell−substrate distance via the CNLS curve fitting of the total impedance (Z_c) to the ECIS model (eq 8) with the *P* weighting in the objective function.

paracellular current traveling horizontally through the cell−substrate gap. The cell−substrate distance of living cells is in the range of 50 nm,[23,24] a distance much smaller than the dimension of cardiomyocytes. Thus, R_g values are highly related to gap dimensions and ion conductivity. However, the ion concentration fluctuation is excluded here because molecular dynamics simulations have demonstrated that fluctuation in ion or solvent particle density occurs only in the interface layer adjacent to regions less than 1 nm thick.[25] Since the existence of R_g is owing to the resistivity of the conductive solution, which obeys Ohm's law, it can be assumed that normalized R_g is inversely proportional to the normalized equivalent cell−substrate distance.

R_g (or reciprocal equivalent cell−substrate distance) change was drawn in the time course after normalization to the initial values (Figure 6b). The normalized resistance rapidly rose to ~184% within the first hour of culture and slowly approached the saturation point of 260% at the end of the first 20 h of culture. Given cardiomyocytes show minor morphological change prior to the dedifferentiation, the change in the cell−substrate gap is mainly distributed by cell adhesion. The cell adhesion initially rose exponentially, reached a plateau, and in the subsequent hours reached saturation.

ECIS Calibration of Cell−Substrate Distance. As described above, EIS data processing of the cell-related impedance can provide the electrical parameters of the cardiomyocyte layer. If the impedance through the body of the cell is considered the capacitive reactance of the cell membrane, all parameters are prepared to calculate absolute cell−substrate distance via advanced analysis of ECIS. However, since no reasonable initial guess is available for the adjustable parameters, the difficulties

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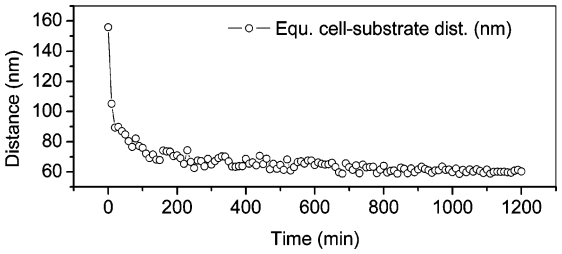

Figure 8. Time course of equivalent cell−substrate distance obtained by calculating the reciprocal of normalized R_g curve followed by the manual calibration of the initial distance with ECIS model. The cell−substrate distance decreases from 155.8 to 60.2 nm within 20 h. The alteration of cell−substrate distance directly describes the adhesion status of cardiomyocytes in primary culture.

in the iteration process will restrict its application in processing isolated data at a single moment. In this research, the advanced ECIS is introduced to calibrate the absolute cell−substrate distance with the manual selection of the initial guess. Combined with the normalized tendency obtained above, the time course of absolute cell−substrate distance can be utilized to record the cardiomyocyte adhesion.

Lo's advanced analysis of ECIS[16] is called in for the calibration, where the geometry of isolated cardiomyocyte is described as a flat rectangular ($L \times W$) box and a half disk ($r_c = W/2$) on each end. In this method, specific impedances of cell-covered (Z_c) and cell-free (Z_n) electrodes are calculated by multiplying the measured impedance by the electrode area. Since the cell membrane principally shows a dielectric nature, the impedance through the cell body is approximately the reactance of C_c in the EIS model, which can be expressed as $Z_m = -j/(\omega C_c)$. One of the two adjustable parameters in Lo's model is the morphological parameter (α) of cell−substrate distance is set as

$$\alpha = \frac{W}{2}\sqrt{\frac{\rho}{h}} \tag{7}$$

where the medium resistivity is ρ and h is the equivalent cell−substrate distance. The other one, R_b, is specific junctional resistances between adjacent cells.

The analytical solution of this method describes the combination of the known impedance data Z_n and Z_m, and two adjustable parameters, α and R_b equals Z_c.[16]

$$\frac{1}{Z_c} = \frac{1}{Z_n + Z_m}$$
$$\left\{1 + \left(\frac{LW}{LW + \frac{\pi W^2}{4}}\right)\frac{\frac{2Z_m}{Z_n}}{\gamma W \coth\left(\frac{\gamma W}{2}\right) + 2.328R_b\left(\frac{1}{Z_n} + \frac{1}{Z_m}\right)} + \left(\frac{\frac{\pi W^2}{4}}{LW + \frac{\pi W^2}{4}}\right)\frac{\frac{2Z_m}{Z_n}}{\frac{\gamma W}{2}\frac{I_0(\gamma W/2)}{I_1(\gamma W/2)} + 1.164R_b\left(\frac{1}{Z_n} + \frac{1}{Z_m}\right)}\right. \tag{8}$$

where $\gamma = [W(Z_n^{-1} + Z_m^{-1})/2]^{-1}$.

The Z_c values were fitted to eq 8 in CNLS curve fitting with the P weighting similar to eq 2. The best fit of ECIS (Figure 7)

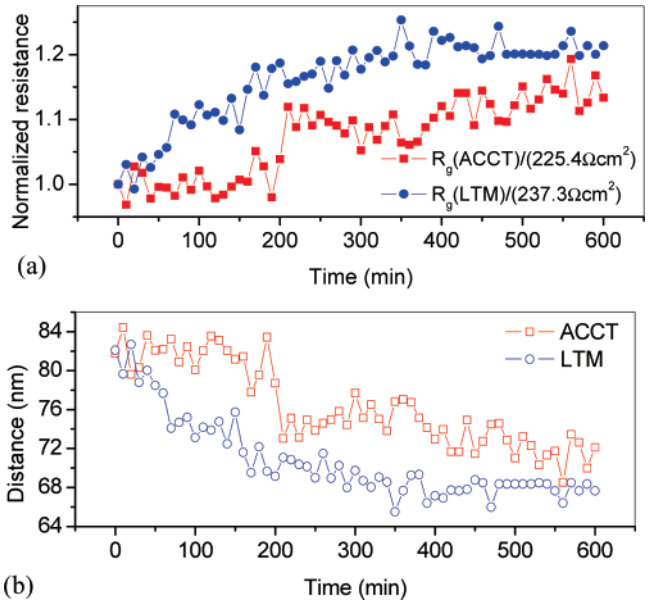

Figure 9. (a) Alteration of normalized R_g in the first 10 h of culture since changing the medium to LTM and ACCT after the initial culture in DMEM for 1 h. The normalizing units are the initial values of R_g in both samples, $R_{g0} = 225.4\ \Omega\cdot cm^2$ for the ACCT sample; $R_{g0} = 237.3\ \Omega\cdot cm^2$ for the LTM sample. (b) The time courses of equivalent cell−substrate distance are obtained by calculating the reciprocal of normalized R_g curve followed by the manual calibration of initial distance with the ECIS model. The initial distances are almost equivalent, cardiomyocytes cultured in LTM exhibit better adhesion than those in ACCT in terms of a smaller cell−substrate distance and a faster adhesion rate. The difference of adhesion in LTM and ACCT is owing to the existence of the growth factors in LTM.

resulted in $\alpha = 6.798\ \Omega^{1/2}\cdot cm$ and $R_b = 0.835\ \Omega\cdot cm^2$. Given the resistivity of DMEM at 37 °C is 72 Ω·cm, the corresponding equivalent cell−substrate distance at the beginning of the initial culture was 155.8 nm, derived via the calculation of eq 7. Once its initial value was calibrated, the whole time course of cell−substrate distance was easily calculated based on the assumption of an inverse proportional relationship between R_g and cell−substrate distance. Figure 8 depicts the alteration of equivalent cell−substrate distance in the initial stage of cell culture, which matches the known cell adhesion process of cardiomyocytes. After 20 h of culture, cardiomyocytes reach a height of 60.2 nm from the laminin substrate, which is consistent with published reports.

Comparison of Cardiomyocyte Culture in ACCT and LTM. The fresh isolated cardiomyocytes were first cultured in DMEM for ~1 h, allowing the initial adhesion of cardiomyocyte to rapidly occur. Afterward, the culture medium was changed to ACCT or LTM. In the primary culture of cardiomyocytes, researchers often use different culture media for specific cell requirements.

As the result of the experiment, the following 10-h culture in ACCT or LTM demonstrated different influences on cardiomyocyte adhesion, as shown in Figure 9a. The increment of normalized R_g of cardiomyocyte culture in LTM is ~20% while that of ACCT is ~15%. Generally speaking, the major difference between ACCT and LTM in our experiment is that LTM carries fetal bovine serum, absent in ACCT. Fetal bovine serum contains multiple growth factors, including insulin-like growth factor I (IGF-I) as a

typical constituent.[26] Targeted to the IGF-I receptor, IGF-I can be used to promote physiologic hypertrophy,[27] accompanied by the activation of focal adhesion kinase[28] in the signaling pathways. The activation of focal adhesion kinase directly indicates the strengthening of cell adhesion.

Calibrated with the advanced ECIS mentioned above, Figure 9b illustrates the alteration of equivalent cell−substrate distance. Despite the fact that initial distances are very close, the LTM sample is more tightly adhered to the substrate than the ACCT sample (68 vs 72 nm). In addition, a comparison of decreasing rate also shows the LTM sample adheres faster than the ACCT sample, indicating LTM promotes cell adhesion more than that of ACCT.

CONCLUSION

In this work, a new sensing and analyzing system is established for the impedance signals in real-time monitoring of cardiomyocyte adhesion to ECM. Though impedance-sensing techniques have been successful with several kinds of biological cells, advanced analysis of pervious impedance sensing is not applicable to cardiomyocyte due to its unknown and variable electrical parameters. The new technique is founded on EIS and experimental evidence, using an equivalent circuit model to describe current pattern on the cell−substrate interface. The new method is convenient for real-time recording the normalized alteration of cell−substrate distance because it enables an automatic data processing with CNLS curve fitting. Simultaneously, specific impedance of the cardiomyocyte layer is worked out for manually calculating the equivalent cell−substrate distance based on ECIS methodology. For the first time, the equivalent cell−substrate distance was recorded in real time and calibrated for the cardiomyocyte culture in time courses. The dynamics processes of initial adhesion and comparison of adhesion rates in ACCT and LTM were recorded and proved the validity of our method of real-time monitoring of cardiomyocyte biological processes.

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ACKNOWLEDGMENT

This work is in part supported by NSF CAREER Award (0239163) and NSF NER Award (0609147). The authors thank Bo Wang, Xu Wang, and other colleagues at Cardiac Muscle Research Laboratory, Brigham and Women's Hospital, Harvard Medical School for their technical advice.

NOTE ADDED AFTER ASAP PUBLICATION

This paper was posted on January 24, 2008. A missing variable superscript was added and the paper reposted on January 28, 2008.

Received for review August 17, 2007. Accepted December 3, 2007.

AC701745C