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Impedimetric quantification of the formation process and the chemosensitivity of cancer cell colonies suspended in 3D environment

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ABSTRACT

In cancer research, colony formation assay is a gold standard for the investigation of the development of early tumors and the effects of cytotoxic agents on tumors *in vitro*. Quantification of cancer cell colonies suspended in hydrogel is currently achieved by manual counting under microscope. It is challenging to microscopically quantify the colony number and size without subjective bias. In this work, impedimetric quantification of cancer cell colonies suspended in hydrogel was successfully developed and provides a quantitative and objective method to describe the colony formation process and the development of colony size during the culture course. A biosensor embedded with a pair of parallel plate electrodes was fabricated for the impedimetric quantification. Cancer cell (cell line: Huh-7) were encapsulated in methyl cellulose hydrogel and cultured to gradually form cancer cell colonies suspended in 3D environment. At pre-set schedule during the culture course, small volume (50 μL) of colonies/MC hydrogel was collected, mixed with measurement hydrogel, and loaded to the biosensor for measurement. Hence, the colony formation process could be quantitatively represented by a colony index and a colony size index calculated from electrical impedance. Based on these developments, chemosensitivity of cancer cell colonies under different concentrations of anti-cancer drug, *i.e.*, doxorubicin, was quantitatively investigated to study the efficacy of anti-cancer drug. Also, dose-response curve was constructed to calculate the IC_{50} value, which is an important indicator for chemosensitivity assay. These results showed the impedimetric quantification is a promising technique for the colony formation assay.

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1. Introduction

Substantial evidences from recent studies showed that solid tumors contain a subpopulation of stem-cell-like cancer cells or tumorigenic cells (Clarke et al., 2006; Jordan et al., 2006; Reya et al., 2001; Dalerba et al., 2007). These cells play an important role in tumor initiation, progression, metastasis, and therapeutic resistance (Costea et al., 2006; Locke et al., 2005). They are often speculated to have several characteristics that make resistant to conventional chemotherapy, including high expression of drug transporters, relative cell-cycle quiescence, high function of DNA repair machinery, and resistance to apoptosis (Dean et al., 2005; Jordan and Guzman, 2004). Recently, studies of cancer stem cells became an attractive area in cancer biology (Clarke et al., 2006; Jordan et al., 2006; Reya et al., 2001; Dalerba et al., 2007). The isolation of cancer stem cells has been successfully achieved by various biological techniques. Sphere culture system was suggested to be one of the efficient approaches in isolating cancer stem cells from solid tumors or cancer cell lines (Lee et al., 2006; Singh et al., 2003). Cancer cells suspended in three-dimensional (3D) hydrogel are cultured to proliferate and differentiate into cancer cell colonies. Soft substrates maintain self-renewal of cancer stem cells but stiff substrates promote differentiation of cancer stem cells (Chowdhury et al., 2010). Therefore, if there are self-renewing stem cells among cancer cells, the sphere culture system might be able to isolate cancer stem cells from a pool of cancer cells. Hence, investigations of the formation process and the chemosensitivity of cancer cell colonies are crucial in cancer research (Liu et al., 2012; Lee et al., 2014; Essig et al., 2014). The colony formation assay became a gold standard for measuring the development of early tumors and the effects of cytotoxic agents on tumors *in vitro*. In order to identify the capability of colony formation and the efficacy of anti-cancer drug, colony number and size are important indexes to be quantified during the culture course. However, conventional bio-assays quantifying indicative cellular components are ineffective especially for large cancer cell

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colonies. Currently, quantification of cancer cell colonies is achieved by manual counting under microscope (Liu et al., 2012; Lee et al., 2014; Essig et al., 2014; Jin et al., 2013). Because cell colonies are suspended in 3D hydrogel and present irregular shapes, this is challenging to microscopically quantify the colony number and size without subjective bias. To tackle this technical hurdle, in this work, impedimetric quantification is proposed to objectively quantify cancer cell colonies in 3D environment during the culture course.

Quantification of cellular responses in conventional two-dimensional (2D) culture model, that cells spread on a flat surface in a monolayer format, was demonstrated by impedance measurement across a pair of planar electrodes (Lei, 2014; Varshney and Li, 2009). If cells adhere and proliferate on the surface of the electrodes, the effective electrode surface area is reduced. Since biological cells are very poor conductors at frequencies below 10 kHz (Ehret et al., 1997), the total impedance across the electrodes is then increased for the determination of the presence of cells. A pioneer work of impedimetric monitoring of cellular behavior was reported in 1984 (Giaever and Keese, 1984). Later, on-line and real-time monitoring of concentration, growth, and physiological state of cells in culture was demonstrated by impedance measurement (Ehret et al., 1997). It showed there was no detectable electrical influence on the cells and the measurement could be used for long-term monitoring. It is generally believed that the impedance measurement can provide a real-time, non-invasive, and label-free monitoring technique for cells. The above studies concerned the quantification of cellular responses in 2D culture format. However, recent studies reported 2D culture model cannot well mimic the native cellular microenvironment because animal cells inhabit 3D environment (Cukierman et al., 2001; Abbot, 2003). Hence, 3D cell culture model that cells are encapsulated and cultured in 3D polymeric scaffold material was proposed (Cukierman et al., 2001; Abbot, 2003). It is regarded as realizing a better approximation of *in vivo* conditions than 2D surfaces and providing a more physiologically-meaningful culture condition for cell-based assays. More recently, impedance measurement of 3D cell culture was demonstrated by gelling a spot of cells–hydrogel mixture (1 μL) on planar electrodes (Jeong et al., 2012). Since there was no accommodating chamber to confine the spot, the spot dimension was varied by number of encapsulated cells and hydrogel density. On the other hand, a perfusion 3D cell culture microfluidic chip was developed to construct a precise, stable, and well-defined culture environment for 3D cell-based assays (Lei et al., 2014). A pair of vertical parallel electrodes located at the opposite sidewalls of the culture chamber was embedded for the on-site impedance measurement. Cells encapsulated in the hydrogel were loaded into the chamber and could receive uniform electric field. Real-time and non-invasive impedimetric monitoring of cell proliferation and chemosensitivity were demonstrated. Based on this development, different electrode configurations were analyzed for maximizing the measurement spatial sensitivity (Canali et al., 2015).

The above excellent developments respectively demonstrated the impedimetric quantification of cellular responses that cells were cultured without forming colonies in 2D or 3D culture environments. However, in cancer research, the colony formation assay is a gold standard for measuring the development of early tumors and the effects of cytotoxic agents on cancer cells *in vitro*. To the best of our knowledge, impedance measurement has not been previously established for the quantification of cancer cell colonies. In this work, we developed a biosensor embedded with a pair of parallel plate electrodes to quantify the cancer cell colonies suspended in hydrogel. A human hepatoma cell line, Huh-7, established from a hepatocellular carcinoma was used in this study because it contains a subpopulation of stem-cell-like cancer cells (Nakabayashi et al., 1982; Zhu et al., 2010; Ma et al., 2007). Cancer cells were encapsulated in methyl cellulose (MC) hydrogel, *i.e.*, soft substrate for isolating cancer stem cells from a pool of cancer cells, and cultured to gradually form cancer cell colonies suspended in 3D environment. In order to quantify the colonies during the culture course, a small volume (50 μL) of colonies/MC hydrogel was collected every 24 h. It was then mixed with measurement hydrogel and the mixture was loaded to the biosensor for impedance measurement. The colony formation process could be quantitatively represented by a colony index calculated from the impedance magnitude. In addition, a colony size index was also developed by analyzing the phase angle of impedance to quantitatively represent the colony size. Based on these developments, chemosensitivity of cancer cell colonies under different concentrations of anti-cancer drug, *i.e.*, doxorubicin, was quantitatively investigated to study the efficacy of anti-cancer drug. Dose-response curve was also constructed to calculate the IC_{50} value, which is an important indicator for chemosensitivity assay. In conclusion, the colony index and colony size index provide quantitative and objective information to describe the cancer cell colonies suspended in 3D environment during the culture course. This technique is proposed to be a promising and powerful tool for colony formation assay.

2. Materials and methods

2.1. Design and fabrication of the biosensor

A biosensor embedded with a pair of parallel plate electrodes was developed for the impedimetric quantification of the cancer cell colonies. The biosensor consisted of 3 layers including an indium tin oxide (ITO) glass layer for common ground electrode, a polydimethylsiloxane (PDMS; Sylgard® 184, Dow Corning, USA) layer for 9 independent cylindrical chambers, and a glass substrate with 9 Cr/Au measurement electrodes. Photographic image of the biosensor is shown in Fig. 1(a). Nine chambers were fabricated on a single biosensor in order to provide high throughput assays. Such that, a pair of parallel plate electrodes was located at the top and bottom surfaces of the cylindrical chamber. The fabrication process of the biosensor is briefly described here. The ITO glass

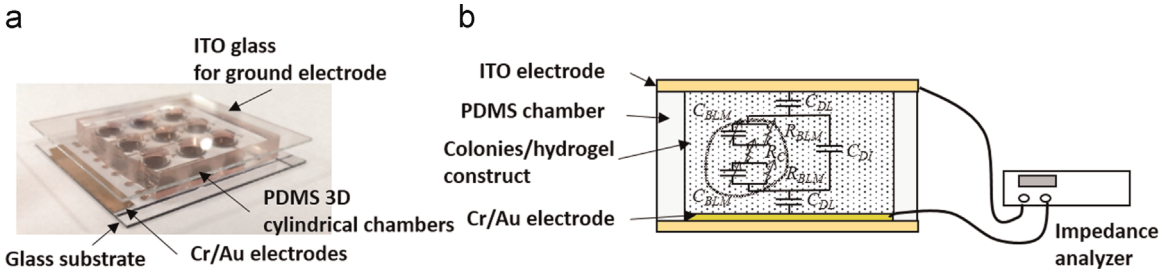

Fig. 1. Design and experimental setup of the biosensor. (a) Photographic image of the biosensor. (b) Illustration of the experimental setup and the equivalent circuit of the cells/hydrogel construct across the parallel plate electrodes.

with the surface resistance of 7–10 Ω was purchased from Uni-Onward Corp., Taiwan. The PDMS layer was fabricated by soft lithography. The Cr/Au measurement electrodes on glass substrate were fabricated by standard microfabrication techniques including metal evaporation, photolithography, and wet etching, respectively. Three layers were washed and sterilized using 70% (w/v) ethanol for 6 h. Next, the glass substrate and the PDMS layer were bonded permanently by oxygen plasma treatment. When performing the quantification, cells/hydrogel mixture was loaded to the cylindrical chamber and the ITO glass layer was then covered on the chamber. Note that there was no bubble remaining in the chamber. An impedance analyzer (Model: VersaSTAT 4, Princeton Applied Research, USA) was connected to the ground and measurement electrodes to perform the impedance measurement. Such that, a uniform electric field was generated by the parallel plate electrodes and penetrated the cells/hydrogel mixture. Cells or colonies were suspended in different locations of the hydrogel could receive identical electric field per unit volume. Because biological cells are very poor conductors, the change of total impedance of the mixture could correlate to the change of cell number or the formation process of cell colonies. Such design provides quantitative and objective information to describe cell proliferation or colony formation process in 3D environment during the culture course.

Electrical properties of cells have been studied to provide the foundation of impedance measurement. Generally, cells have membrane capacitance of 0.5–1.3 μF/cm^2, membrane resistance of 10^2–10^5 Ω cm^2, and low cytoplasmic resistance (Pethig, 1979). That indicated cells are very poor conductors compared with the culture medium. Equivalent circuits of the impedance measurement of cellular behavior in 2D and 3D environments were respectively described (Varshney and Li, 2009; Lei et al., 2014). Illustration of equivalent circuit of the cells/hydrogel construct across the parallel plate electrodes is shown in Fig. 1(b). Cells are represented by a lumped component with membrane capacitance, C_{BLM}, membrane resistance, R_{BLM}, and cytoplasmic resistance, R_C. Also, a dielectric capacitance of hydrogel, C_{DI}, is introduced in parallel with the lumped cell component. C_{DL} is double layer capacitance induced on the electrode surface. Both C_{DI} and C_{DL} are regarded as constants. In the case of cells suspended in hydrogel, the total impedance of the cells/hydrogel construct can describe the cellular behavior only when the impedance of hydrogel is enough high. If the impedance of hydrogel is too low compared with the impedance of the lumped cell component, it dominates the total impedance and cellular behavior cannot be determined. For example, impedance of the MC hydrogel is too low and the total impedance is not sensitive to the cellular behavior. In our studies, agarose hydrogel was selected to be the measurement hydrogel because it has an appropriate impedance (Lei et al., 2014). Therefore, cells are regarded as a lumped electrical component and the change of cellular behavior can be determined by the total impedance of the cells/hydrogel construct across the parallel plate electrodes.

2.2. Cell culture

Human hepatoma cell line, Huh-7, was used and was kindly provided by Prof. I-Chi Lee at Chang Gung University. Culture medium was Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS; Invitrogen, USA) and antibiotic/antimycotic (100 U/mL of penicillin G sodium, 100 mg/mL of streptomycin, and 0.25 mg/mL of amphotericin B; Gibco-BRL Life Technologies, USA). Cells were amplified by standard cell culture technique and trypsinized using 0.05% trypsin for 3 min, centrifuged at 1000 rpm for 5 min, and resuspended in the medium for further experiments.

2.3. Formation of cancer cell colonies and exposure of colonies to anti-cancer drug

Cancer cells were encapsulated in MC hydrogel and cultured in standard 24-wells microplate for the formation of cancer cell colonies. MC hydrogel is a kind of soft substrates for 3D cell culture. It was prepared by dispersing the MC powder (Methocel® MC; Sigma, USA) in phosphate-buffered saline (PBS; 50 mM phosphate, 150 mM NaCl, and 10 mM EDTA; pH 7.6) at a concentration of 1.8% (w/v) at room temperature. Then, it was sterilized by using autoclave at 121 °C under 100 kPa for 20 min for further use. In order to eliminate cell seeding on the bottom surface of the wells, a layer of agarose hydrogel (low-gelling temperature agarose; Sigma, USA) (mixture of 150 μL 2% (w/v) agarose solution and 150 μL 2 × DMEM) was first coated on the bottom surface of the wells. Next, after solidification, a mixture of 250 μL 2 × DMEM with cells at the cell number of 30×10^3 and 250 μL MC hydrogel was loaded into the well and cultured in a 37 °C, humidified atmosphere, and 5% CO_2 incubator (Model: 370; Thermoscientific, USA). Cancer cells were suspended in MC hydrogel and gradually formed colonies during the culture course of 7 days.

Doxorubicin is an effective anti-cancer drug to treat a wide range of cancers; but, serious side effects are also induced (Tao et al., 2007). Because of high drug resistance of cancer stem cells (Dean et al., 2005; Jordan and Guzman, 2004), investigation of chemosensitivity of cancer cell colonies is important for precise control of cancer chemotherapy. In this study, cancer cells were suspended in MC hydrogel and formed colonies with certain number and size in the beginning 3 days of the culture course. Then, starting from day 3, 100 μL culture medium containing doxorubicin (Sigma, USA) at desired concentration was applied to the well every 24 h. Doxorubicin inhibits cancer cell migration and also induces cell apoptosis (Tanada et al., 2012; Chen et al., 2011). Cell colonies shrank and the level of shrinking depended on the concentration of doxorubicin.

2.4. Impedance measurement of cancer cell colonies

Impedance measurement of cancer cell colonies suspended in 3D environment was performed every 24 h during the culture course. 50 μL colonies/MC hydrogel was collected from the well and then mixed with 50 μL measurement hydrogel, *i.e.*, 1% (w/v) agarose hydrogel. Next, 20 μL mixture was loaded to the biosensor for impedance measurement. Potential of 0.1 Vrms was applied across the parallel plate electrodes and the impedance was measured from 50 Hz to 50 kHz. The duration of each measurement required around 30 s. To quantitatively describe the colony formation process, we defined a colony index which is the impedance change at the measurement frequency of 100 Hz. In addition, a colony size index represented the colony size was also defined by analyzing the phase angle change of impedance at the measurement frequency of 100 Hz.

3. Results and discussion

3.1. Optimization of the measurement conditions

The measurement conditions, including the chamber height of the biosensor and the measurement frequency, were investigated in order to optimize the linearity and the sensitivity of the impedance measurement of cells/hydrogel construct. Cells at different cell numbers were encapsulated in the measurement hydrogel, *i.e.*, 1% (w/v) agarose hydrogel. Then, cells/hydrogel mixtures were respectively loaded into the cylindrical chambers with different heights. Impedance measurement was performed to study the

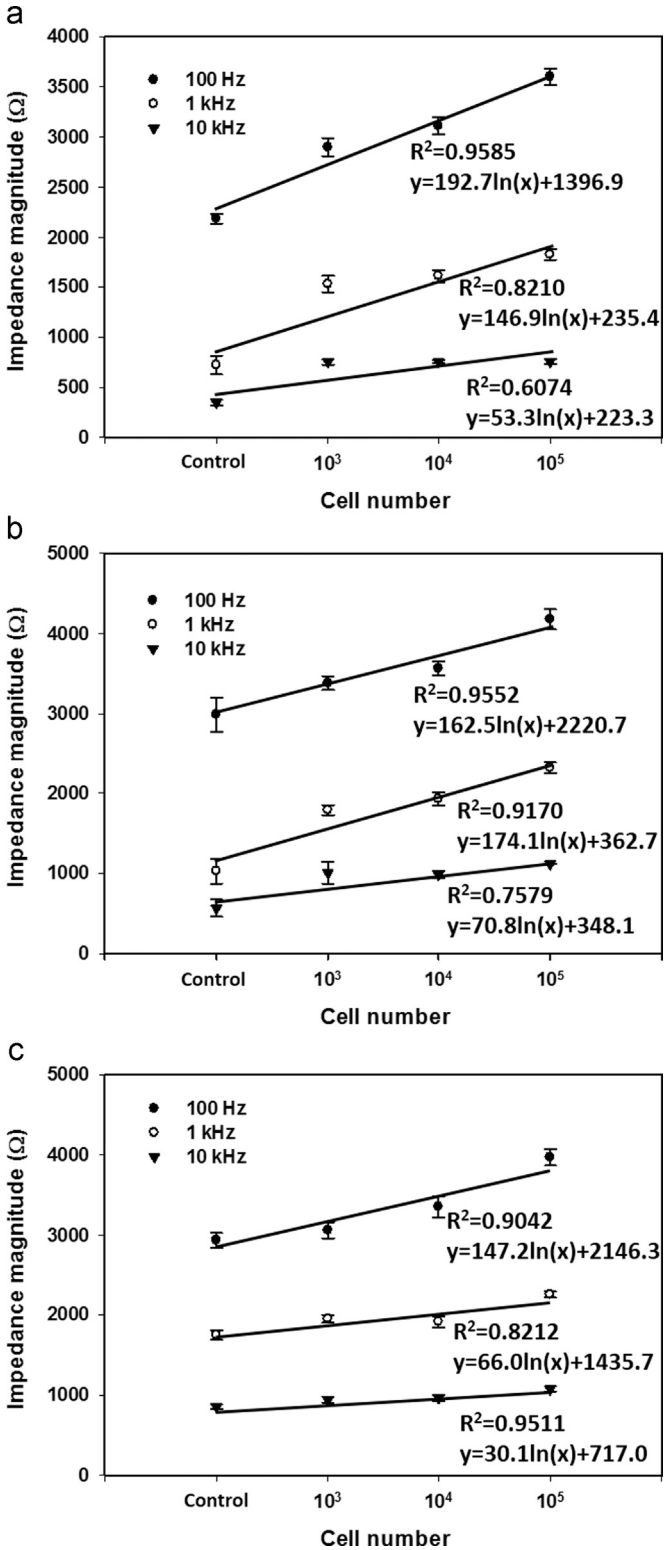

Fig. 2. Correlations between cell number and impedance magnitude measured at 100 Hz, 1 kHz, and 10 kHz under the biosensors with different chamber heights of (a) 4, (b) 6, and (c) 8 mm. Error bars represent the standard deviations of 3 repeated measurements.

electrical characteristics of the 3D cells/hydrogel construct. Because formation of colonies cannot be controlled, colonies with controlled number and size cannot be harvested. In this optimization study, cells were suspended in the hydrogel without forming colonies. Correlations between cell number and impedance magnitude measured at 100 Hz, 1 kHz, and 10 kHz under the biosensors with different chamber heights of 4, 6, and 8 mm are shown in Fig. 2. Their corresponding *R*-squared values and the curve fitting equations are also shown. Results revealed that the highest *R*-squared value and sensitivity were obtained in the 4 mm height chamber at the measurement frequency of 100 Hz. Generally, linearity and sensitivity increased with the decrease of the chamber height. Higher signal-to-noise ratio could be obtained because higher electric field was generated by shorter distance between 2 parallel plate electrodes. Because cytoplasmic resistance is low, measurement at low frequency, *i.e.*, 100 Hz, could enhance the influences from the impedance of cell membrane capacitance and resistance in parallel to the total impedance of the cells/hydrogel construct. The effect was gradually diminished when the measurement frequencies increased, *i.e.*, 1 and 10 kHz. Therefore, impedance measurement at 100 Hz using the biosensor with 4 mm height chamber can well describe the presence of cells in 3D environment.

3.2. Quantification of the formation process of cancer cell colonies

Cancer cells growing in 3D culture format are more resistant to cytotoxic agents than in 2D culture format (Dean et al., 2005; Torisawa et al., 2005). Currently, the colony formation assay is a gold standard for measuring the development of early tumors and the effects of cytotoxic agents on tumors *in vitro*. In this assay, cancer cells in the size of around 10 μm proliferate and form cancer cell colonies with irregular shapes in the size of over 100 μm. A sequence of microscopic images capturing the formation process of cancer cell colonies is shown in Fig. 3(a). Both number and size of the cancer cell colonies gradually increased during the 7 days culture. It is difficult to objectively count the number and size of the colonies suspended in 3D hydrogel under microscope. Especially after day 4, colonies were fused with each other and irregular huge colonies were observed. In such situation, impedimetric quantification of the cancer cell colonies was performed to provide objective information. The Bode plot of the colonies/hydrogel construct during the culture course is shown in Fig. 3(b and c). Based on the analysis of the impedance magnitude, colony index was calculated and plotted in Fig. 3(d). The colony index showed gradually increase with the increase of the number and size of colonies (shown in Fig. 3(a)) and provided a quantitative index to describe the colony formation process. On the other hand, by analyzing the impedance phase angle, colony size index was calculated and plotted in Fig. 3(e). From day 0 to day 3, the phase angles at the measurement frequency of 100 Hz were around −60°. Colony size was small (shown in Fig. 3(a)) and the electric field could still penetrate the colonies. The total impedance of the cells/hydrogel construct showed capacitive effect mainly contributed by cell membrane capacitance. Starting from day 4, colonies gradually became large (shown in Fig. 3(a)) and insulated, *i.e.*, resistive effect. Capacitive effect in the total impedance of the cells/hydrogel construct gradually reduced. This was observed from the phase angles at the measurement frequency of 100 Hz, *i.e.*, around −30°. Therefore, colony size index was proposed to represent the development of the colony size during the culture course.

3.3. Validation of the impedimetric method

Microscopic counting of the colony number and size is generally used and is a gold standard of quantification in the colony formation assay (Hernanda et al., 2013; Sarveswaran et al., 2015; Zhang et al., 2015). Due to different perceptions from personnel, the results from microscopic counting are subjective. In our study, colony number was counted based on the colonies that were

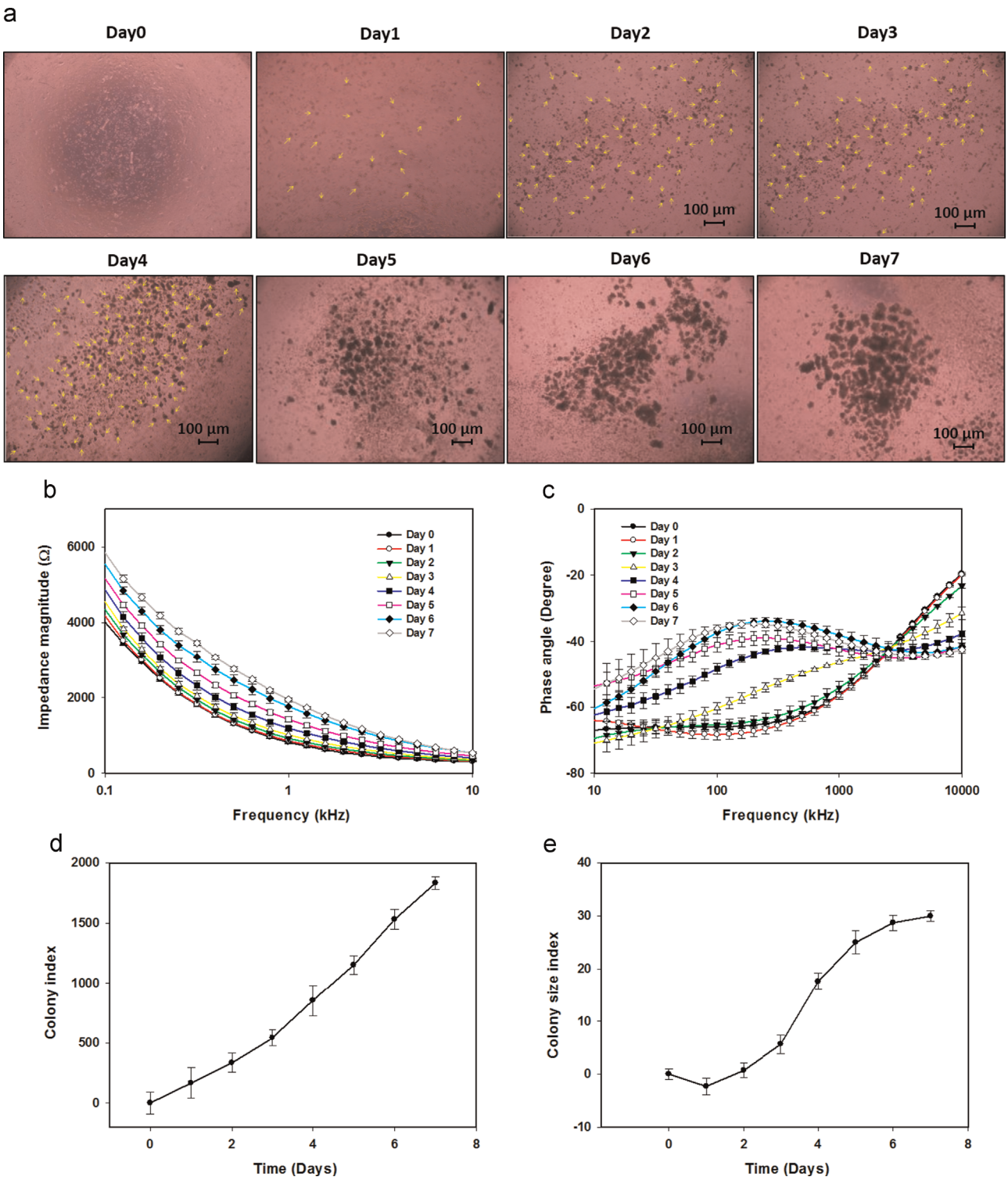

Fig. 3. Quantification of the colony formation process based on impedimetric method. (a) A sequence of microscopic images capturing the formation process of cancer cell colonies up to 7 days. Yellow arrows indicate colonies that are larger than 50 μm in diameter. (b, c) Bode plot of the colonies/hydrogel construct during the 7 days culture course. (d) Colony index calculated from impedance magnitudes at the measurement frequency of 100 Hz. It provides a quantitative index to describe the colony formation process. (e) Colony size index calculated from impedance phase angles at the measurement frequency of 100 Hz. It quantitatively represents the development of the colony size during the culture course. Error bars represent the standard deviations of 3 repeated measurements. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

larger than 50 μm in diameter. However, colonies tended to fuse together and became irregular shapes after day 4 (shown in Fig. 3 (a)). Manual counting may not be possible to isolate each colony and colony number cannot be determined after day 4. Quantification of colony number based on microscopic counting up to 4 days is shown in Fig. 4(a-1). In addition, colony size was also measured and quantification of colony size based on microscopic counting up to 7 days is shown in Fig. 4(a-2). Since the colonies fused together after day 4, merged colonies were excluded in the measurement. Although microscopic counting was influenced by

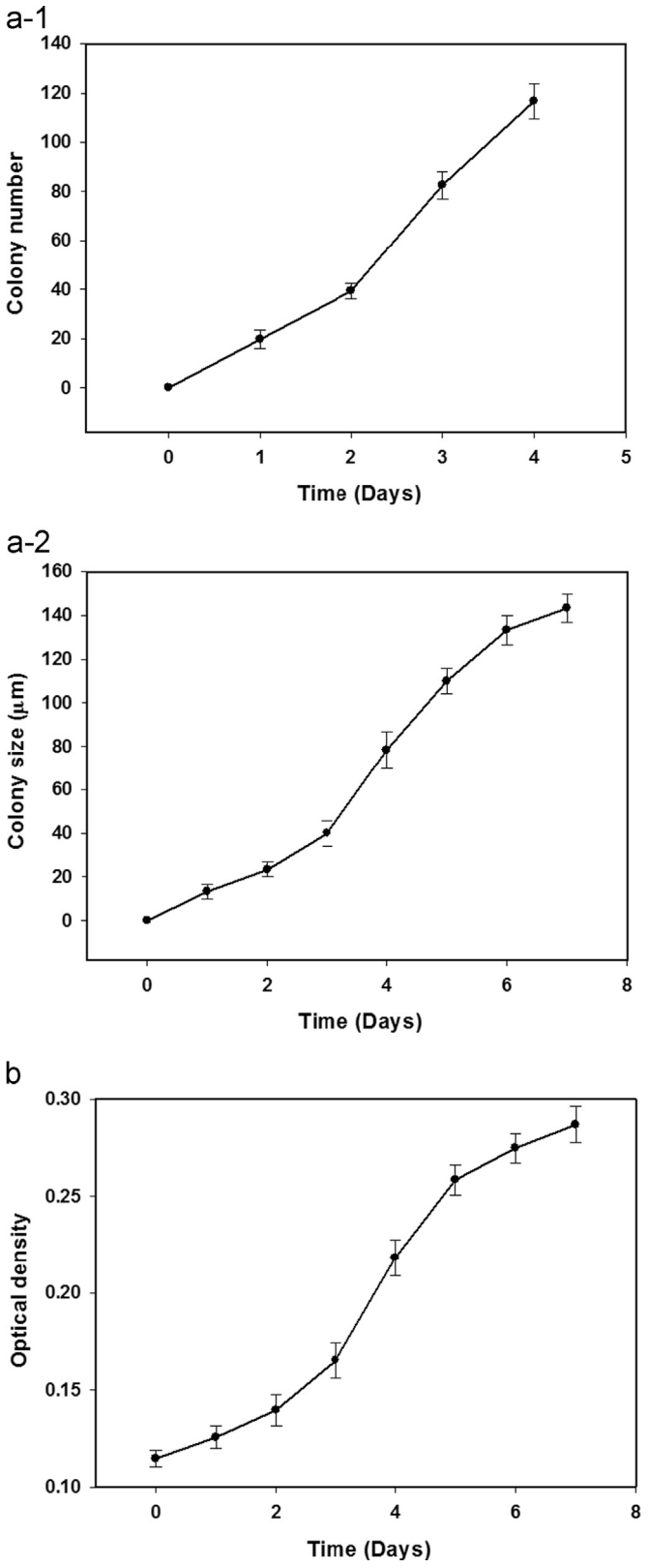

Fig. 4. Quantification of the colony formation process based on conventional methods. (a) Quantification of (a-1) colony number and (a-2) size based on microscopic counting. (b) Quantification of individual cell number broken from colonies based on bio-assay.

personnel perception, colony number and size were shown monotonic increase during the culture course. Results were aligned with the colony index and colony size index (shown in Fig. 3(d and e)).

Quantification of cell number based on bio-assay is generally used in cell-based assay. Bio-assay is to analyze the indicative cellular components and the result is objective. For quantification of cell colonies, colonies are broken up into individual cells and bio-assay is used for quantification in some research groups (Lee and Chang, 2015; Yang et al., 2013). In our study, WST-1 assay (Roche Applied Science, USA) was used to quantify the number of individual cells broken from colonies every 24 h. Because the reagent of WST-1 assay reacts with the respiratory chain of the mitochondria, *i.e.*, only active in metabolically intact cells, the optical density of the reacted product directly correlates to the number of viable individual cells. Cancer cells were encapsulated in MC hydrogel and cultured in standard 24-wells microplate for the formation of cancer cell colonies. At the preset schedule, 50 μL colonies/MC hydrogel was collected from the well and colonies were then broken up into individual cells by manual pipetting. Next, 15 μL solution containing individual cells was mixed with 35 μL culture medium. Then, 5 μL reagent of WST-1 assay was applied to the mixture and incubated for 2 h. The optical density was read on a plate reader at an absorbance of 440 nm with a reference wavelength of 660 nm. Quantification of the colony formation process based on bio-assay is shown in Fig. 4(b). Results showed that the growth rate of individual cells became slow after day 4. It was because the colonies became large (shown in Fig. 3 (a)) and insufficient nutrient and oxygen were supplied for the growth of individual cells, especially cells in the central of colonies. In addition, colonies tended to fuse together and larger colonies were formed. However, bio-assay can only quantify the number of individual cells and cannot represent the size of colonies. The result of bio-assay mistakenly implied the growth of colonies became slow. In contrast, colony index (shown in Fig. 3(d)) represented a growth index of colonies combining the information of colony number and size, which is necessary for the investigation of the development of early tumors.

3.4. Quantification of the chemosensitivity of cancer cell colonies

Impedimetric quantification of the chemosensitivity of cancer cell colonies was demonstrated and colony index and colony size index were used to quantitative describe the colony formation process under the stimulation of anti-cancer drug. Cancer cells were suspended in MC hydrogel and colonies were gradually formed in the beginning 3 days of the culture course. Then, starting from day 3, culture mediums containing doxorubicin at different concentrations of 10, 50, and 100 μg/mL were respectively applied to the wells every 24 h. Different concentrations of doxorubicin induced different levels of shrinking of cancer cell colonies. Chemosensitivity of the colonies under different concentrations of anti-cancer drug could be observed from the microscopic images, as shown in Fig. 5(a). On the other hand, impedimetric quantification of the cancer cell colonies was concurrently performed to provide objective information, as shown in Fig. 5(b and c). Results revealed that colony index could well describe the chemosensitivity of cancer cell colonies. Dynamic response of the colonies after drug application and concentration-dependent effect at the end-point of the assay were represented by the colony index during the culture course. Also, colony size index could characterize the development of colony size that is an important information for the colony formation assay. In addition, the IC_{50} value of a drug was determined by constructing a dose-response curve shown in Fig. 6. The IC_{50} value of doxorubicin applied to cancer cell colonies was calculated to be 67 μg/mL. In this study, stem-cell-like cancer cells isolated by sphere culture system were used for the chemosensitivity assay. Higher drug resistance was shown compared to cells cultured in conventional

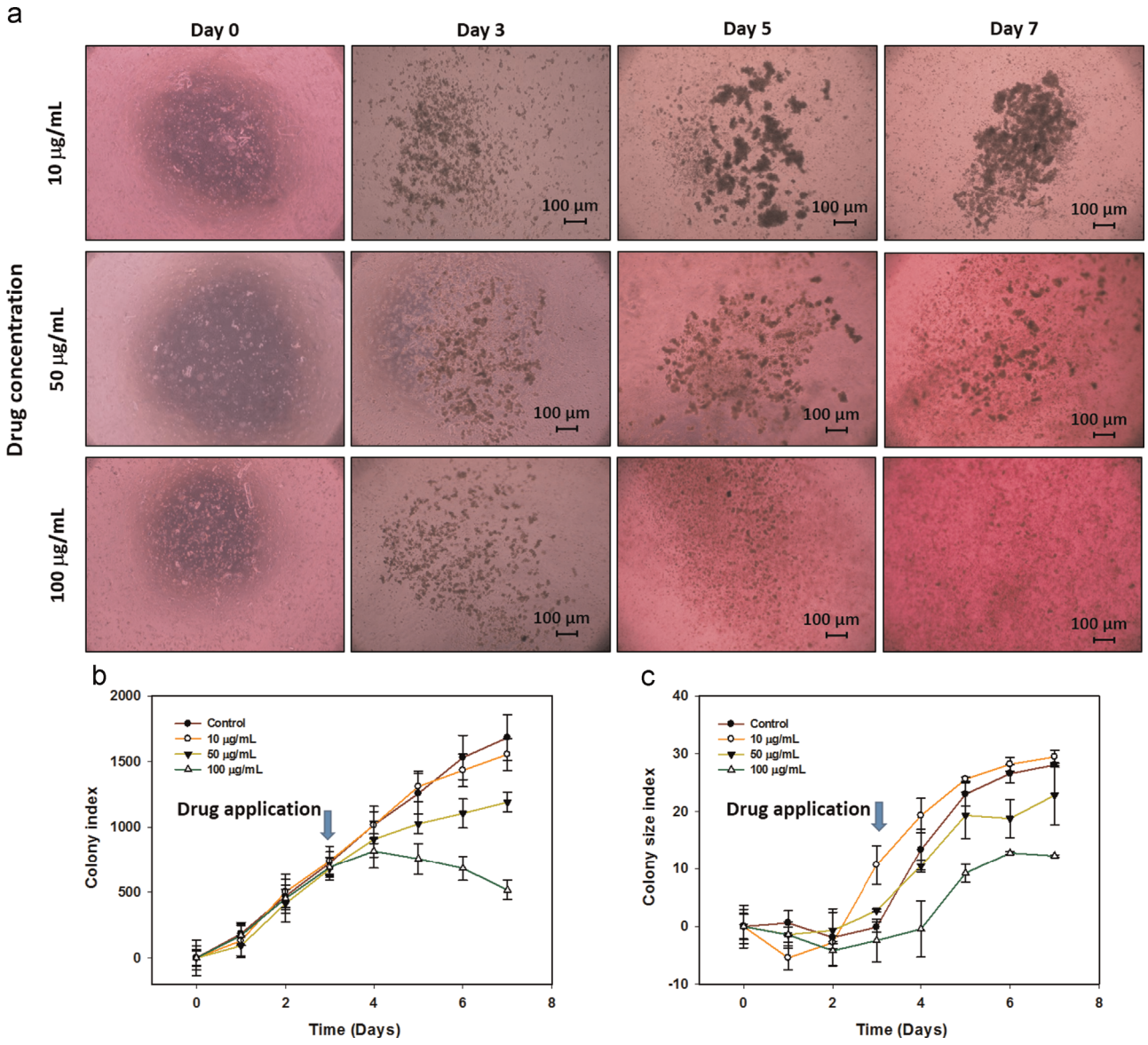

Fig. 5. Quantification of the chemosensitivity of cancer cell colonies under anti-cancer drug at different concentrations of 10, 50, and 100 μg/mL. (a) Microscopic images of cancer cell colonies captured at day 0, 3, 5, and 7. (b) Impedimetric quantification of the chemosensitivity of cancer cell colonies represented by colony index. (c) Impedimetric quantification of the colony size represented by colony size index. Error bars represent the standard deviations of 3 repeated measurements.

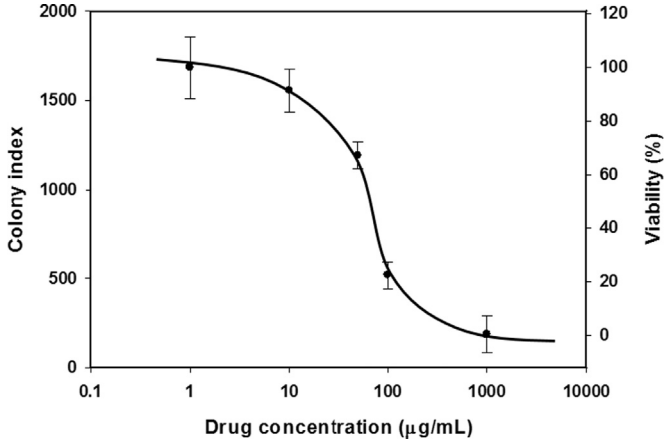

Fig. 6. Dose-response curves of cell viability according to drug concentrations. Error bars represent the standard deviations of 3 repeated measurements.

2D culture environment (Fornari. et al., 2010; Lee et al., 2002). That agreed with the previous studies that stem-cell-like cancer cells are more resistant to cytotoxic agents (Dean et al., 2005).

In pharmaceutical research, validation of potential drug candidates is based on cell-based assays in the initial stage of drug discovery. High physiologically relevant culture environment can increase the predication accuracy of clinical response to drug compounds. That can shorten the drug development cycle and then decrease the development cost. Our work suggests an efficient and simple method for quantification of cancer cell colonies objectively. Results demonstrated the feasibility for chemosensitivity assay in the investigation of cancer cell colonies to drug compounds.

4. Conclusion

Impedimetric quantification of cancer cell colonies suspended in hydrogel has been demonstrated by using a biosensor

embedded with a pair of parallel plate electrodes. Colony index and colony size index were introduced to quantitatively describe the colony formation process and the development of colony size during the culture course. Hence, dose-response curve of cancer cell colonies to drug compounds could be constructed to calculate the IC_{50} value, which is an important indicator for chemosensitivity assay. This technique provides a quantitative and objective method to analyze the development of early tumors and the effects of cytotoxic agents on tumors *in vitro*. Reliable assessment with accurate information is very important for understanding the cancer pathogenesis and thus developing effective therapeutic strategies.

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