Plastic pillar inserts for three-dimensional (3D) cell cultures in 96-well plates

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ABSTRACT

We have developed a plastic pillar insert to facilitate miniaturized three-dimensional (3D) cell cultures in 96-well plates by forming 3D hydrogel droplets containing cells (about 1 μL) on the tip of the pillar insert. Hemispherical 3D droplets containing cells were formed simply by immersing the tip of the pillar insert into a mixture of poly-L-lysine (PLL) and BaCl₂ in a cell-encapsulation apparatus, followed by dipping it into cell suspension in alginate. Compared to traditional 3D cell culture platforms such as polymer scaffolds in 96 wells and 3D hanging drop plates, it allows us to easily change cell growth media or expose 3D cells to reagents by immersing the tip of the pillar inserts in different 96 wells filled with growth media or reagents. As a proof of concept, A549 and PC9 cell lines from human non-small cell lung cancer (NSCLC) were grown on the pillar insert and tested for cytotoxicity with Erlotinib. Both cells on the tip of the pillar insert grew over time, forming 3D structures unlike traditional 2D cell monolayer cultures and mimicking in vivo-like cellular microenvironment. The number of cells in alginate droplets was linearly proportional to the cell seeding density. The doubling time of A549 and PC9 cells were 15.9 h and 16.1 h, respectively, which were similar to those obtained from traditional 2D cell cultures. IC₅₀ values from A549 and PC9 cells exposed to Erlotinib for 3 days were 15.2 ± 7.0 μM and 1.7 ± 0.4 nM, respectively, indicating that PC9 cells with EGFR mutation are highly sensitive to Erlotinib. Interestingly, the IC₅₀ value of 3D PC9 cells grown on the pillar insert was 6 times lower than those obtained from 2D PC9 cells grown on the surface of 96-wells, whereas the IC₅₀ value of 3D A549 cells were 5 times higher than those from 2D A549 cells. The result may represent that A549 and PC9 cells grown on the pillar insert are better mimicking what happens in humans.

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1. Introduction

Cell-based assays have been used extensively for in vitro compound efficacy/toxicity testing and, indeed, have become routine in drug discovery processes [1]. Established in vitro cell models adopted in the 96- and 384-well micro-titer plate with liquid-dispensing and plate-handling robotics as the standard workhorse for high-throughput screening (HTS) provide valuable preliminary insight for drug candidates by testing the bioactivity of a series of compounds and identifying potential toxic liabilities to humans. For example, US National Cancer Institute 60 human tumor cell lines (NCI60) have been used to rapidly identify compounds with higher efficacy against particular tumor types such as leukemia, colon, lung, central nerve system (CNS), renal melanoma, ovarian, breast, and prostate. Human cells grown on the surface of 96- or 384-wells, known as two-dimensional (2D) cell monolayer cultures, have been used as the gold standard for the past few decades in the drug discovery processes [1]. However, many cells of normal and malignant origin lose some of their phenotypic properties when grown in vitro as 2D monolayer [2–5]. The formation of tissue-like structures is highly inhibited in 2D monolayer cultures due to the strong affinity of cells to most artificial surfaces and the restriction to a 2D space, severely limiting intercellular contacts and interactions. Compared to 2D monolayer cell cultures, some researchers have already reported that 3D treated cells show different morphologies [6], and protein/gene expressions [7,8], and drug responses [9,10]. Therefore, in recent years, there have been enormous efforts toward three-dimensional (3D) cell cultures [11,12] that can maintain specific biochemical and morphological features of human cells similar to the corresponding tissues in vivo, including human cells grown within the 3D structure of hydrogel such as alginate, Matrigel, and collagen or human cells grown on 3D polymer scaffolds in 96 well plates [7–13]. Recently, 3D cell cultures were demonstrated in 3D hanging drop plates by aggregating cells in hanging droplets by gravity, eventually leading to the formation of...
spheroids [13]. These 3D cell culture approaches facilitate cell–cell interactions, such as those between stromal and tumor cells for cancer growth and progression studies, and enhance cell-to-cell or cell-to-extracellular matrix (ECM) adhesion/signaling, ultimately leading to the expression of phenotypic proteins/gens and the formation of in vivo tissue-like morphology. For example, fibroblasts cultured on 2D surfaces formed a flat shape, strikingly dissimilar from the bipolar/stellate shape found in tissues, whereas fibroblasts cultured in 3D collagen structure showed a more in vivo tissue-like phenotype [13,14]. The profile of gene expression in melanoma cells grown on 2D surfaces and in 3D hydrogel indicated that 173 genes related to chemokines, laminin, and c-Jun were strongly up-regulated in 3D [15,16]. Similarly, the expression profiles of vascular smooth muscle cells showed that 100 genes were differently up-regulated in 3D compared to those in 2D [17]. In addition, C4-2B prostate cancer cell lines cultured in hyaluronic acid (HA) hydrogel showed lower resistance to a cytotoxic quinoline alkaloid, camptothecin, than their counterpart cultured on 2D surfaces [10]. In contrast with C4-2B cells, HepG2 hepatoma cell lines in 3D demonstrated higher resistance to cisplatin by forming multicellular spheroids compared to those grown in 2D [18].

To facilitate 3D cell cultures in hydrogel on the traditional well plate platforms, it is important to aspirate and dispense a mixture of cell suspension and viscous hydrogel as big as 10–100 µL with a liquid dispenser and change cell growth media over time without damaging the 3D structure of hydrogel containing cells, which is a difficult and daunting task. To alleviate these problems and facilitate 3D cell cultures at a minimum assay volume as small as 1 µL, we have developed a plastic pillar insert combined with the conventional 96-well plates (Fig. 1). The alginate droplets containing cells were generated on the pillar insert by immersing the tip of the pillar insert into a trench containing a mixture of poly-L-lysine (PLL) and BaCl₂ in a cell-encapsulation apparatus, followed by dipping it into another trench containing cell suspension in alginate. This method allowed us to generate uniform cell-containing hydrogel droplets on the pillar insert without using a liquid dispenser. In addition, encapsulated cells were exposed to fresh growth media or compounds by simply transferring and immersing the pillar insert from column of a well to another column of that well without damaging the 3D structure of hydrogel. Compared to our previous miniaturized 3D-cell culture platform constructed on functionalized glass slides [19,20], our new platform is highly compatible with conventional cell-based assays using 2D cell monolayers, as the pillar insert can be combined with 96-well plates with cell-staining dyes. As a proof of concept, A549 and PC9 cell lines from human non-small cell lung cancer (NSCLC) were encapsulated in alginate on the pillar insert and tested with Erlotinib for cytotoxicity. Erlotinib is a drug used to treat NSCLC and several other types of cancer, and it is a reversible tyrosine kinase inhibitor, acting on the epidermal growth factor receptor (EGFR). Thus, Erlotinib is known to work more effectively on the PC9 cell line, which has EGFR mutation [21,22].

2. Materials and methods

2.1. Fabrication of a plastic pillar insert

A pillar insert and a cell-encapsulation apparatus were prepared by cutting cyclic olefin copolymer (COC) with a computer numerical control (CNC) machine. COC was selected because of its high transparency, excellent biocompatibility, and adequate stiffness for physical machining. A single pillar insert consists of eight pillars, each pillar being 2 mm in diameter and 9 mm in height, and 9 mm pillar-to-pillar distance, and thus, compatible with the conventional 96-well plates (Fig. 2A). The cell-encapsulation apparatus has a pair of grooves to help guide the pillar insert and a long and narrow trench (5 mm in width, 70 mm in length, and 0.8 mm in height) to accommodate reagents for encapsulating cells in hydrogel. Prior

![Fig. 1. Schematics of 3D cell cultures in hydrogel: (A) conventional 3D cell cultures in the 96-well plate and (B) new 3D cell cultures constructed on the pillar insert combined with the 96-well plate.](image1)

![Fig. 2. Schematics of the pillar insert (A) and the cell-encapsulation apparatus (B).](image2)
to using the pillar inserts and the cell-encapsulation apparatus, the pillar inserts and cell-encapsulation apparatus were immersed in 70% ethanol for 30 min for sterilization followed by complete drying at room temperature.

### 2.2. Cell culture and the preparation of cell suspension

A549 and PC9 cell lines from NSCLC and cultured in Roswell Park Memorial Institute (RPMI) medium 1640 (CellGro) supplemented with 10% fetal bovine serum (FBS from CellGro) and 1% penicillin–streptomycin (Invitrogen) in cell-culture Petri dishes (from Corning, 100 mm in diameter) in a humidified 5% CO2 incubator (Sheldon Mfg, Inc.) at 37 °C. The cells were routinely passaged every 2–3 days at 80% confluence. The cell suspensions were prepared by trypsinizing a confluent layer of the cells with 0.3 mL of 0.25% trypsin–0.53 mM EDTA (Invitrogen) from the culture dish and resuspending the cells in 7 mL of 10% FBS-supplemented RPMI. After centrifugation at 2000 rpm for 3 min, the supernatant was removed and the cell precipitates were resuspended with 10% FBS-supplemented RPMI to a final concentration of 10 × 10^5 cells/mL. The number of cells in RPMI was calculated by using the automatic cell counting kit of AccuChip (Digital Bio). For the passage of the cells, 5 × 10^5 cells were seeded in a 100 mm Petri dish containing 20 mL of growth medium. In addition, the passage number of the cells used in the experiment was between 20 and 30.

### 2.3. Encapsulation of cells in alginate on the pillar insert

The cells were encapsulated in alginate droplets and strongly attached on the tip of the pillar insert through physical affinities between COC to Matrigel and Matrigel to PLL and ionic interactions between positively-charged PLL and negatively-charged alginate (Fig. 3). BaCl₂ was added to form a gel by cross linking between carboxylic groups on alginate. The bottom adhesion layer on the pillar insert was prepared by adding 600 µL of Matrigel in the trench of the cell-encapsulation apparatus and immersing the tip of the pillar insert into the Matrigel solution. In this step, for preventing Matrigel from gelling, the trench is placed on ice to keep temperature under 4 °C. Following the drying of the Matrigel in room temperature, the tip of the pillar insert was immersed in 600 µL of a mixture of PLL and BaCl₂ in the trench.

After the complete drying of PLL–BaCl₂, the tip of the pillar insert was immersed into cell suspension in 1% alginate, immediately separated from the cell-encapsulation apparatus, and then stored in a Petri dish for 1 min at room temperature, resulting in the gelation of the alginate matrix on the PLL–BaCl₂ layer. Immediately after gelation, the pillar insert with cells in alginate was immersed in the growth medium in a column of 96 wells for cytotoxicity assays. With this approach, the encapsulated cells can be exposed to fresh growth media, compounds, or staining dyes by simply transferring and immersing the tip of the pillar insert from one column of 96 wells to another column of 96 wells without damaging the 3D structure of alginate (Fig. 4). Matrigel and low viscosity algic acid from Macrocystis pyrifera were purchased from BD biosciences and Sigma–Aldrich, respectively. Matrigel was used without dilution and 2% (w/v) alginate solution was prepared in sterile de-ionized distilled water prior to further dilution with cell suspension. The PLL–BaCl₂ mixture was prepared by mixing 100 µL of 0.05 M BaCl₂ (Sigma–Aldrich) with 200 µL of 0.01% PLL (Sigma–Aldrich). A suspension of cells in alginate was prepared by mixing equal volumes of cell suspension in 10% FBS-supplemented RPMI (2 × 10^6 cells/mL) with 2% alginate solution in distilled water so that the final concentration of cells and alginate were 1 × 10^5 cells/mL and 1%, respectively. While changing the cell seeding density, the final alginate content remained constant at 1% throughout the experiment. The cell-encapsulation apparatus was reused after cleaning with 70% ethanol. To visualize cells
encapsulated in alginate droplets under a fluorescent microscope (OLYMPUS BX51), the pillar insert containing cells were stained with 200 μL of 1 μM calcein AM (Invitrogen) dissolved in a saline solution containing 140 mM NaCl and 20 mM CaCl2 for 30 min in 96 wells. Green dots represent live A549 cells in alginate on the tip of the pillar insert (Fig. 5). The picture was taken at 12.5× magnifications with a 490 nm excitation filter and a 520 nm emission filter.

2.4. Cytotoxicity tests with Erlotinib

As a proof of concept, A549 and PC9 cell lines grown in alginate droplets were tested with Erlotinib (Tarceva, BioVision) for cytotoxicity, and the results were compared with their 2D counterparts. A compound stock solution was prepared by dissolving Erlotinib in dimethyl sulfoxide (DMSO) at 20 mM. To maintain a final DMSO content of less than 0.5% and to prepare 50 μL of test compound solutions at 200-fold higher concentrations than the desired final concentration, the compound stock solution was 5-fold serially diluted in DMSO (7 dosages plus 1 control). As a control, 100% DMSO without compound was used. To prepare 200 μL of further diluted test compound solutions in a 96-well plate, 1 μL of compounds diluted in DMSO was mixed with 199 μL of the growth medium before use. Therefore, the final concentrations of Erlotinib used were 0, 0.064, 3.2, 16, 80, 400, 2000, and 10000 nM, respectively. Due to potential compound carry-over, compound solutions were added from low to high concentration in the 96-wells. Prior to drug exposure, the pillar insert containing cells was incubated in the growth medium in 96 wells for 1 day to maintain high cell viability. After cells were exposed to Erlotinib at different concentrations for 3 days, the remaining cell viability was measured by incubating cells for 1 h in 200 μL of a CCK-8 solution (Dojindo Molecular Technologies, Kumamoto, Japan) and reading absorbance at 450 nm with a plate reader (SpectraMax190, Molecular Devices). To avoid any interference by the pillar insert with the absorbance measurement of the CCK-8 solution, the pillar insert was removed from the 96-well plate prior to the measurement. The CCK-8 solution was prepared by mixing 20 μL of CCK-8 with 180 μL of the growth medium. The data were plotted with SigmaPlot 10 and GraphPad Prism 4 to measure cell growth rates and obtain sigmoidal dose response curves and IC50 values.

3. Results and discussion

The pillar insert was designed to perform high-throughput 3D cell cultures in the 96-well plate and allowed us to test the cytotoxicity of compounds. The alginate droplets containing cells on the tip of the pillar insert were securely attached to the surface by forming a gel (Fig. 5). In addition, the cells in alginate droplets formed multi-cellular aggregates during proliferation, which are morphological characteristics of cultured 3D cells (Fig. 5C). The encapsulated cells were easily exposed to growth media, compounds, or staining dyes by transferring and immersing the pillar inserts into the 96 wells (Fig. 4). To demonstrate the feasibility of the pillar insert for 3D cell cultures and cytotoxicity assays, we measured the uniformity of the alginate droplets containing cells and the growth rates of A549 and PC9 cells on the pillar inserts by using a CCK-8 assay kit. Finally, the cytotoxicity of Erlotinib was tested using the pillar insert containing A549 and PC9 cells in alginate droplets.
cycle. As shown in Fig. 6, absorbance values obtained from alginate droplets containing live A549 and PC9 cells were linearly proportional to the seeding density of cell suspensions. The calibration curves after liner regression showed that \( R^2 \) values for A549 and PC9 cells are 0.997 and 0.999, respectively, indicating that there is a good correlation between absorbance and the cell seeding density. In addition, the slopes for A549 and PC9 cells were \( 3.14 \times 10^{-2} \) and \( 6.48 \times 10^{-5} \) absorbance/cell, respectively. Using the slopes from the calibration curves and the volume of one droplet on the pillar insert, we were able to calculate the number of A549 and PC9 cells in alginate droplets by measuring absorbance.

3.2. Growth rates of A549 and PC9 cells in alginate droplets on the pillar inserts

The growth rates of cells encapsulated in alginate droplets on the tip of the pillar insert were determined and compared with those from 2D cell monolayers. As shown in Fig. 7, A549 cells were grown in alginate droplets as evidenced by increased green fluorescence over time, forming unique 3D aggregates.

The cells were stained with calcein AM, which can be transported through the cellular membrane and can produce a green fluorescent response from living cells, making it useful for testing cell viability. The images of A549 cells in Figs. 5 and 7 were taken by a traditional fluorescent microscope, and there was no unusual fluorescence or reflection observed from the cells on the pillar insert. In addition, it was easy to monitor cell growth on the pillars over time under a bright field microscope as well because of highly transparent COC used. We believe that the imaging of various cells on the pillars in general are as simple as conventional ways of cell imaging because numerous high-throughput cell imaging and analysis systems including celloomics have a function of automatic focus on cells built in already for high-content screening. Therefore, it will not be a problem to obtain images of cells on the pillar insert where the cells are no longer in one plane but the pillars may be slightly higher or lower in the 96-well plates. To determine cell growth quantitatively, we measured the absorbance of A549 and PC9 cells at a 1 million cells/mL seeding density (i.e., 1000 cells/droplet seeding density) over time using the CCK-8 solution (Fig. 8). The same numbers of cells were seeded but grown on two different systems, namely 3D cultures on the pillar insert and 2D cultures on the surface of 96 wells, both with 200 \( \mu \)L of the growth medium. As shown in Fig. 8A, both A549 and PC9 cells in alginate droplets grew over time on the pillar inserts, and the growth rates
were gradually reduced, presumably due to the limited space available for cell growth within alginate droplets. Interestingly, A549 and PC9 cells grown on the surface of 96 wells did not show such retardation in growth (Fig. 8B), indicating that there is still enough space for these cells to grow in 96 wells for up to 5 days. This disparity in growth can be overcome by increasing the diameter of the pillar insert or reducing the cell seeding density so that cells can grow linearly for a longer period of time without space limitation. In addition, we have not observed A549 and PC9 cells migrated out of the alginate gel (anionic polysaccharide gel) even after 8 days of culture on the pillar insert, indicating that cells may not be able to escape from the alginate gel and the space limitation in the alginate gel affects their growth. However, numerous cells secreting matrix metalloproteinases (MMP) may be able to migrate out of gel matrices when these cells are encapsulated in protein-based gels such as collagen and Matrigel and grown on the pillar insert over the long period of time. Due to robust surface chemistries, the cell-containing alginate gels at the tip of the pillars were strongly attached for over 10 days of cell cultures in RPMI. To avoid any destabilization of the alginate gel by chelating agents such as a high concentration of phosphate or Tris buffer in cell growth media, we implemented much stronger cross linker of negatively charged alginate such as BaCl2 instead of CaCl2. The doubling time of A549 and PC9 cells was calculated from absorbance values measured between 1 and 3 days presented in Table 1. For both A549 and PC9 cells, the doubling times obtained from 3D and 2D systems were similar, which were comparable with those from the literature.

### 3.3. Cytotoxicity of Erlotinib tested with A549 and PC9 cells grown in alginate droplets on the pillar insert

The applicability of the pillar insert combined with the 96-well plate for cell-based assays was tested with A549 and PC9 cells exposed to Erlotinib. Erlotinib was selected as it is a well-known inhibitor of the epidermal growth factor receptor (EGFR), specifically targeting EGFR tyrosine kinase, which is overexpressed and occasionally mutated in various types of cancer. Thus, EGFR-mutated cell lines including PC9, HCC827, and H3255 cells are known to show a great response to Erlotinib (i.e., extremely low IC50 values were obtained) [21,22]. A549 cells and EGFR-mutated PC9 cells both from NSCLC were selected as model cell lines for cytotoxicity tests. A 3D cell culture system was demonstrated with A549 and PC9 cells grown in alginate droplets on the pillar insert, whereas a 2D cell culture system was prepared in 96 wells with monolayers of A549 and PC9 cells for comparison. For both systems, the seeding number of A549 and PC9 cells was 1000 cells in an alginate droplet and the 96 wells. After incubation in the cell growth medium for 1 day to maintain high cell viability prior to drug exposure, Erlotinib with 7 dosages plus 1 no-drug control was added to the 96-well plates. After 3 days of incubation in the presence of the drug, the viability of A549 and PC9 cells in 3D and 2D systems was determined by using the CCK-8 solution. Dose response curves and IC50 values were generated from absorbance reading at 450 nm and all data were obtained from three separate runs. As expected, the dose response curves obtained from A549 and PC9 cells were very different from each other (Fig. 9). For both 3D and 2D cell culture systems, IC50 values of PC9 cells were dramatically lower

![Fig. 8](image1.png)

**Fig. 8.** The growth curves of A549 and PC9 cells in alginate on the pillar inserts (A) and in the 96-well plate (B).

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<thead>
<tr>
<th>Cell lines</th>
<th>Doubling time [h]</th>
<th>IC50 values</th>
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<td>2D cell monolayers in 96 wells</td>
<td>3D cell cultures on the pillar inserts</td>
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<td>A549</td>
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<td>PC9</td>
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![Fig. 9](image2.png)

**Fig. 9.** Dose response curves of Erlotinib with A549 and PC9 cells cultured in 3D alginate droplets on the pillar inserts and 2D monolayers.
than those from A549 cells, indicating that Erlotinib is highly selective for EGFR-mutated cell lines (Table 1). To further analyze the experimental results statistically, P values were calculated by one-way ANOVA (analysis of variance) between IC50 values of A549 and PC9 cells cultured in both 2D and 3D systems. As a result, the P values of IC50 values between A549 and PC9 cells were less than 0.05, indicating that the toxic responses of Erlotinib to A549 and PC9 cells in two different cell culture systems are statistically different. In addition, the IC50 values obtained from the 2D cell-culture system were similar to those from the literature, representing that the cytotoxicity experiments were carried out properly. Interestingly, IC50 values from 3D A549 and PC9 cells were 8700-fold different whereas those values from the 2D counterparts were only 310-fold different. The IC50 value of 3D PC9 cells grown on the pillar insert was 6 times lower than that obtained from 2D PC9 cells grown on the surface of 96-wells, whereas the IC50 value of 3D A549 cells was 5 times higher than those from 2D A549 cells. This kind of disparity on IC50 values between 3D and 2D cell-culture systems has been already reported in the literature and is considered as a distinctive characteristic of cells cultured in 3D systems [11, 12, 18]. We hypothesized that the expression of phenotypic proteins/genes and the formation of in vivo tissue-like morphology facilitated by cell–cell or cell–ECM interactions in the 3D cell-culture system are responsible for significant difference in IC50 values.

4. Conclusions

We have successfully demonstrated that the pillar insert combined with the traditional 96-well plate can be used for 3D cell cultures. The simple method developed for cell encapsulation allowed us to grow A549 and PC9 cells in 1 μL of alginate droplets on the tip of the pillar insert. The cells were grown on a pillar insert immersed in the growth medium in 96 wells, forming unique 3D structures over time. The number of cells encapsulated in alginate droplets was uniform among droplets prepared at the same condition and was linearly proportional to the seeding density. In addition, the cytotoxicity of Erlotinib tested on 3D A549 and PC9 cells showed that the response of the drug on EGFR-mutated PC cells was highly selective, consistent with the results obtained from the literature. With the pillar insert/96-well plate platform allowing us to encapsulate cells in alginate by simple immersion and expose live 3D cells to various reagents by easy movement, we envisage that traditional 2D cell monolayer-based assays will be much easier replaced with 3D cell-based assays. Finally, we believe that the pillar insert/96-well plate platform represents a major breakthrough in 3D cell cultures by allowing us to perform miniaturized cell-based assays as small as 1 μL. It will be particularly beneficial when precious primary human cells are used for toxicity assays.

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References


Biographies

Dong Woo Lee received a B.S. degree in the School of Medical Engineering at Pusan National University in 2000, a M.S. degree in Department of Biosystems at Korea Advanced Institute of Science and Technology (KAIST) in 2004, and a Ph.D. degree in the Department of Bio- and Brain Engineering at KAIST in 2009. From
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**Bosung Ku** received his Ph.D. degree in the Department of Chemical and Biological Engineering from Rensselaer Polytechnic Institute in 2006 and has focused on microscale enzymatic reaction and cell-based assay that can be applied to the field of drug screening. Since September 2006, he has been working at Samsung Electro-Mechanics Co., Ltd., as a senior engineer and project leader. His group is mainly developing microarray chip platforms and related automatic Microsystems that are now intensively evaluating from Samsung Medical Center and global big pharmaceutical companies.

**Jhingook Kim** received his M.D. degree from School of Medicine at Seoul National University in 1983. He had trained as board-certified thoracic and cardiovascular surgeon at Seoul National University Hospital. And he had surgical fellowship training in Mayo Clinic and research fellowship training in MD Anderson Cancer Center. Since December 1994, he is working at Samsung Medical Center, Sungkyunwan University School of Medicine as a professor. He is taking care of more than 400 thoracic surgical cases including lung cancer annually and has studying for the personalized cancer care based on the cancer genomics and patient-derived primary cancer cells.

**Moo-Yeal Lee** received his Ph.D. degree from KAIST in 1999 and has focused on developing highly automatable, microarray biochip platforms that can adequately mimic human metabolism and assess cell-specific toxicity of new chemical entities and their metabolites. Specific areas of current research include enzyme-, carbohydrate-, virus-, and cell-based microarrays. Since early 2002 at Rensselaer Polytechnic Institute and later at Solidus Biosciences, Inc., Dr. Lee has been making important contributions to many interdisciplinary applications of microarray biochips, including the proprietary metabolizing enzyme toxicology assay chip (MetaChip), data analysis toxicology assay chip (DataChip), P450 inhibition chip, multienzyme lead optimization chip (Multizyme Chip), heparin glycan chip (Hep-Gly Chip), metabolic stability assay chip (MesaChip), transfected enzyme and metabolism chip (TeamChip), and RNA interference chip (RNAi chip). He believes that microarray chip platforms as well as microsystems represent a promising, high-throughput microscale alternative to conventional in vitro multi-well plate platforms and create new opportunities for rapid and inexpensive assessment of ADME/Tox at very early phases of drug development.