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A Portable Microfluidic Device for Rapid Diagnosis of Cancer Metastatic Potential with Programmable Modules of Temperature and CO_2

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If metastasis of lung cancer can be found and treated early, a victim might have an improved chance to prevail over it, but routine examinations such as chest radiography, computed tomography and biopsy cannot characterize the metastatic potential of lung-cancer cells; critical diagnoses to define optimal therapeutic strategies are thus lost. We designed a portable microfluidic device for rapid diagnosis of cancer metastatic potential. Featuring a micro system to control temperature and a bicarbonate buffered environment, our device discriminates a rate of surface detachment as an index of migratory ability of cells cultured on pH-responsive chitosan. We labeled metastatic subpopulations of lung cancer cell lines, and verified that our device is capable of separating cells according to their metastatic ability. As only few cells are needed, a patient's specimen from biopsies, e.g. fine-needle aspiration, can be processed on site to offer immediate information to physicians. We expect that our design will provide valuable information in pre-operative evaluations so as to assist to define therapeutic plans for lung cancer, as well as for metastatic tumors of other types.

Introduction

Lung cancer is the foremost cause of cancer mortality worldwide, accounting for 1.3 million deaths annually.[1] The 5-year survival rate of lung cancer is only about 15 %, mostly because lung cancer has a strong tendency to metastasize at an early stage,[2] causing its bad prognosis with a large rate of mortality. Although lung cancer might be initially detected on imaging morphological changes inside the lungs and confirmed with a tissue biopsy, routine diagnoses such as chest radiography, computed tomography and biopsy do not provide direct information on the metastatic potential, as this requires technical challenges to assess the function of living specimen at a small size. Through the concept of *lab on a chip*, here we explored the advantages offered by microfluidic manipulation to design a device for cancer metastatic potential MD-CaMP, which enables bedside fractionation of minute mass specimens. MD-CaMP features programmable modules of temperature control, bicarbonate-dependent pH regulation, and a pH- responsive chitosan coating to discriminate the cancer metastatic potential through the absorption of fibronectin.

Animal cell cultures are widely used in *ex vivo* assay systems for biomedical application; the ability to control precisely the extracellular conditions at a physiologically meaningful environment is primordial for establishing cell-based models.[3] Since the cell samples of concern often consist of heterogeneous populations, to understand information about individual cells, one requires to separate cells of varied types to obtain single-cell fractions.[4] Conventional techniques to sort cells, including selective enzyme sensitivity, differential centrifugation, and selection medium culture, are limited by purity, yield and cell viability; they are tedious and require advanced skill of personnel. Label-assisted methods such as flow cytometry or magnetic beads absorption are highly specific methods, but both require a further process to remove the labels.[5] Microfluidics has the potential to reform the way in which we are accustomed to work in cell biology. Microfluidics enables a design flexibility, great reproducibility, fluidic control,[6, 7] diminished consumption of reagents and chemical waste, abbreviated duration of sample treatment, selective gas permeability and experimental feasibility. Several microfluidic platforms to separate cells include those based on size-based cell capture and separation, on-chip DEP, cell-affinity micro-chromatography, and magnetically activated micro-sorting.[4] Nevertheless, most of these studies are focused on the manipulation of cells in suspension, such as lymphocytes and circulating tumor cells,[8] rather than adherent cells.[9] The design of a microfluidics system that permits the individual manipulation and functional evaluation on adherent cells thus constitutes one of the most attractive challenges of the field.

To attain proper physiological environment in an *ex vivo* assay system, most of the laboratory practices utilize microscope incubators to control three important factors -- temperature, CO_2 and moisture. A commercial biological microscope incubator currently takes tens of minutes to reach a steady environment; it is also expensive and energy-consuming, and a long-term observation might cause stage deformation and result in the loss of optical focus.[10]

Microfluidic devices can be an effective solution because of its possibility to integrate precise and rapid control of their temperature[10, 11] and CO_2;[12, 13] in the case of temperature control, various configurations of a microheater have been extensively discussed. Also, CO_2 chips with a hot plate as the source of heat, have also been constructed and proven to maintain normal morphology and behaviour of cells for a longer period of time than those without a CO_2 supply.[12]

Hydrogels are polymers swollen with water; some of their properties are altered with external stimuli such as pH, temperature, solvent, ionic strength, light, electric and magnetic fields, or chelating species.[14] Chitosan, a pH-responsive natural cationic polysaccharide well known because of the protonation-deprotonation equilibrium of the primary amine on its glucosamine residues, is one of many interesting polymers derived from renewable resources, and is suitable for cell screening. The isoelectrical point of chitosan occurs at pH 7.4;[15] the pK_a of chitosan is about 6.1-7.0.[16, 17] On the surfaces of tissue culture grade polystyrene, it can be coated as a thin film with the characteristics of an intelligent surface, which is able to alter its absorption to fibronectin and thus lead to cell detachment at differential degrees.[18,19] Fibronectin, an extracellular matrix glycoprotein, has been reported to be up-regulated in advanced stage lung cancer cells, and the metastatic potential of lung cancer is positively correlated to the amount of fibronectin expression.[20, 21] Higher production of fibronectin is not only an hallmark of cancer cell mesenchymal epithelial transition,[22] it can also relay intracellular signalling pathways that lead to increased aggressiveness,[23] and is also an important factor for the "seed and soil" process for distant lung cancer metastasis;[24] thus, fibronectin upregulation can be considered as one of the most reliable markers for lung cancer metastatic potential.

In this article, we propose the design of a microfluidic device for cancer metastatic potential MD-CaMP, which enables bedside fractionation of minute mass specimens. MD-Camp features programmable modules of temperature control, bicarbonate-dependent pH regulation, and a pH- responsive chitosan coating to discriminate the cancer metastatic potential through the absorption of fibronectin. It provides a controlled temperature and partial pressure of CO_2 to cells with no requirement of a cell-culture incubator, and permits direct cell imaging through a conventional optical microscope. As the absorption of fibronectin on pH-dependent chitosan can result in differential rates of detachment of the cells upon variation within a physiologically permissible range of environmental pH, we exploited that a specimen obtained from a biopsy might be assessed for fibronectin expression as a functional discrimination of the migratory ability of cancer cells, provided that the design of the assay device is small enough to handle the few cells obtained. In this article, we demonstrate that we can fractionate cells according to their migratory ability, with no enzymatic treatment. Our design constitutes the first example that integrates control of both temperature and CO_2 for the maintenance and functional discrimination of a biopsy-size living specimen in a microfluidic chip.

Materials and methods

Microfluidic chip design and fabrication

Figure 1(a) presents a schematic illustration of our integrated microfluidic device containing a cell-culture chamber, a CO_2 chamber, a micro-heater and a micro thermal sensor. As shown in Fig. 1(b), the microfluidic chambers were fabricated on poly(dimethylsiloxane) (PDMS) using soft lithographic methods. The master mould was fabricated on a polymethylmethacrylate (PMMA) plate using a computer numerical control (CNC) machine (EGX-400, Roland Inc., Japan) equipped with a 0.5-mm drill bit at a feed speed 7 mm/s and a rotational rate 26,000 rpm. PDMS containing the prepolymer and the curing agent at the mass ratio 10:1 was mixed, degassed in vacuum, poured into the PMMA mould and cured at 80 ℃. The PDMS structure was treated with an oxygen plasma before bonding and assembly.

As shown in Fig. 1(c), a grid of platinum micro-heater is fabricated on glass to control precisely and rapidly the temperature based on the feedback signal of a designed micro thermal sensor and a control program. The grid-type micro-heater and micro thermal sensor were fabricated with standard lithography and lift-off.[25] The bio-compatible soda-lime glass (thickness 0.7 mm) was first cleaned with piranha solution (H_2SO_4:H_2O_2 = 3:1). A positive photoresist (AZ 4620, Clariant Inc., USA) was then spin-coated (3000 rpm, 40 s) and patterned with photolithography to define the micro-heaters and the micro thermal sensor of temperature. With E-beam evaporation, we deposited a Pt layer (120 nm) above a Cr layer (30 nm) on the glass substrate (0.7 mm). The Pt layer was then patterned with a standard lift-off process to obtain the micro-heaters and the microthermal sensor.

The assembled chip is show in Fig. 1(d).The area of cell culture was set to be 15 mm x 1 mm, perfused with culture medium through the inlet and outlet holes (diameter 0.5 mm). The CO_2 chamber was filled with $NaHCO_3$ solution (750 μL, 10 mM in deionized water). The pH of the bicarbonate-buffered cell culture medium was adjusted through the amount of CO_2 penetrating a gas-permeable PDMS wall; the partial pressure of CO_2 was regulated by the concentration of $NaHCO_3$ filled in the CO_2 chamber surrounding the cell-culture chamber. Cells grew on a pH-responsive chitosan surface that enabled differential detachment of cells according to their relative levels of fibronectin expression. In addition, the cells were isolated and collected without using protease-based solutions.

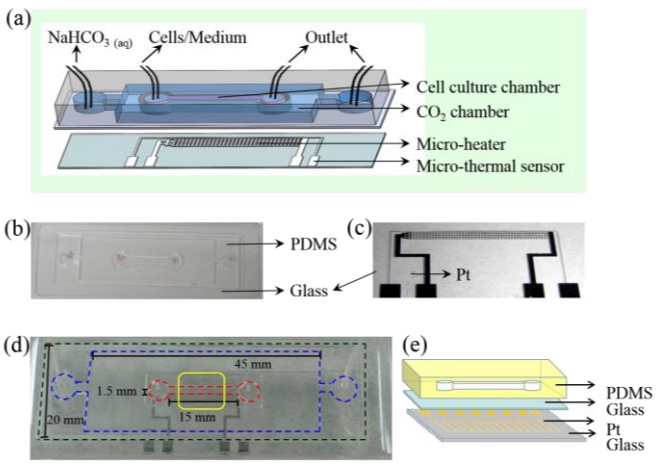

Figure 1. Design and fabrication of the microfluidic chip. (a) Schematic illustration of a micro cell-culture chip. (b) CO_2 and cell-culture chambers. (c) Pt micro-heater. (d) Top view of an assembled device. The red dashed line indicates the cell-culture area and between the blue and red lines is the CO_2 chamber. The area marked by the yellow square box is the region for observing the cell behaviour. (e) Side view of the material of each part. The transparent PDMS facilitates cell observation.

Cell lines, media and reagents

Cells were maintained at 37 ºC in a humidified air atmosphere with CO_2 (5 %) and passaged by conventional tryptic digestion. RPMI 1640 medium, MitoTracker Red CM-H2XRos, Vybrant CFDA SE Cell Tracer, trypsin, EDTA, certified grade fetal bovine serum (all from Invitrogen, USA), H33342 (Merck, USA) and other reagents (Sigma, Gilingham, UK) were obtained from the indicated suppliers.

The human lung-cancer cell line CL1 was established from a man (age 64 years) with a poorly differentiated adenocarcinoma.[23] Subpopulations (sublines CL1-0, CL1-1 and CL1-5) from CL1 cells were selected according to their differential invasiveness through a Boyden chamber, and sibling cell clones were assigned according to the number of rounds through Boyden chamber selection that each has passed. To verify the authenticity of the cell clones in our hands, we performed wound closure assay to reconfirm the differential degree of migratory ability as documented. Wound closure assay is performed by restrained scratch on a nearly confluence culture of cells, to create uniform cell-free spaces that allows adjacent cells to migrate into the area. We measured the original width of the area immediately after the scratch as 100%, and for the subsequent time points, the very same locations were measured again to obtain the remaining cell-free space percentage that have not been migrated by adjacent cells. The micrographs shown on Figure 2(a) are phase contrast illumination images, where the central dark areas are the scratched cell free zones, whose remainder across the time are

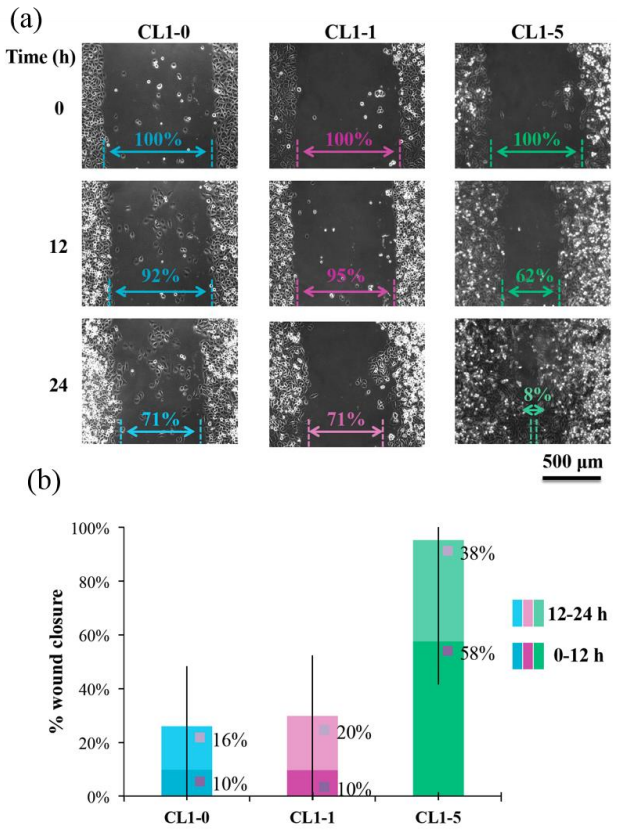

Figure 2. Migratory ability of CL1 lung-cancer cells. (a)Time-lapse images of live CL1 sublines CL1-0, CL1-1 and CL1-5 were obtained with a phase-contrast microscope after the indicated intervals upon standard wound-scratch procedures. (b) Mean wound closure fractions (as percentiles) from three independent experiments.

Chitosan substrate was prepared from chitosan solution (0.5 mL, 0.25 % mass/mass) dissolved in acetic acid (3 %, mass/mass). Chitosan substrate (30 μL) was added into microchannels to fill them and was dried at 60 ºC for one day to form a thin film. These microchannels were then exposed to ultraviolet light overnight and washed thoroughly with PBS before cell seeding.

Cell seeding and detachment assay

CL1 cells were seeded into chip from a solution ($5x10^5$ cells/mL) and cultured (pH 7.4 for 16 h). In co-culture experiments, CL1-1 cells and CL1-5 were prestained with 5-μM MitoTracker Red CM-H 2XRos (red) and Vybrant CFDA SE Cell Tracer (green), respectively, for 15 min at 37 ºC and mixed in ratio 1:1 to seed into the microchannel. For flushing, a micro-flow syringe pump served to infuse continually the corresponding medium so as to mimic the rate, 8 mm/s, of blood flow in arteries.

Detection systems

For analysis of the detachment of fluorescent cells, a semi-automatic inverted microscope (Zeiss D1) was used for direct observation of the microchannel. Phase-contrast imaging at magnification 400x was used to monitor the cell condition and seeding density; DIC at magnification 1000x was used for morphological analysis of the cell after adjustment of the culture pH. An infrared thermal imager (FLIR A325sc) was used to confirm the effectiveness of the chip with temperature feedback.

Results and discussion

Control of temperature with the grid-micro-heater

We fabricated a Pt micro-heater on glass and controlled precisely the temperature of the culture area with the assistance of the micro thermal sensor of temperature and the feedback-control program. With the micro thermal sensor, the curve of metal resistance versus temperature was first measured for the calibration and then used in the feedback-control program (LabVIEW). The voltage applied to the micro-heater was adjusted based on the resistance mismatch between the measured and the target temperatures. When the target temperature was set as 37 ℃, in PID controllers, to determine the appropriate feedback control coefficient (K_p = 0.01, T_i = 0.1), the overshoot of the micro-heater temperature produced a maximum temperature 40.6 ℃, and remained stable at 37± 0.3 ºC within 1 min. The multi-physics coupling-simulation software (COMSOL) simulated the bottom temperature of the glass substrate (thickness 0.7 mm), the current flow through the Pt grid of the micro-heater of thickness 120 nm, line width 50 μm, and each grid area 250 μm × 250 μm. By passing the fixed current density at the electrode and letting the current flow through constructed metal-grid geometry, we can manipulate the resistance heat to raise or decrease the flow temperature. Considering the glass heat transfer, thermal overlay and heat loss, we derived the surface distribution of temperature of the glass wafer with a micro-heater, shown in Figure 3 (a). To simulate the real temperature status in a cell-culture channel, which is the site of cell adhesion and growth, we added a glass slide as a layer above the micro-heater chip. From the simulated result (Fig. 3(a)) and the infrared thermal image (Fig. 3(b)) from experiment, we confirmed that the distribution of temperature in the area of cell growth (within the yellow frame of Fig. 3(b)) was uniform.

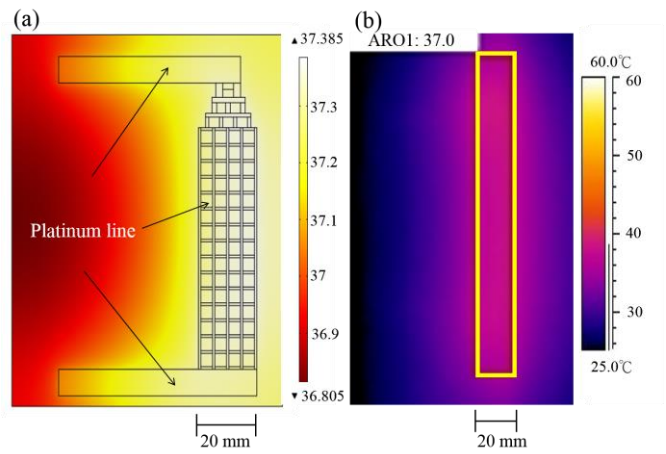

Figure 3. Temperature distribution of a Pt micro-heater generated by (a) simulation and (b) detected with an IR imager. The area marked by the yellow box represents the region of cell-culture maintained at average temperature 37 ℃ during the experiment.

Our micro-heater has the following main features. (1) The two-dimensional heat conduction is even; there is a decreased disadvantage that the heat generated with the thin metal wire is not readily conducted horizontally, which might produce an abrupt regional decrease of temperature; unlike a heater of array type or of serpentine shape, it has no significant temperature gradient on the boundary.[23] (2) Fabrication is simple -- its manufacturing is simpler than that of a fence-like or array-like heater;[25] the fabrication of a heater of array type with self-compensation is difficult, requiring deposition of two metals of distinct densities, such as gold and platinum.[22] (3) The temperature of the heater is precisely controlled with a programmable PID when the external environment varies; compared with a heater of block type or of serpentine shape, or of array type without self-compensation, our heaters of array type with self-compensation provide the best performance of a heat source. [25] (4) The size is accurately estimated: during the observation of cells, the heating wires serve as the reference grids to count the cell numbers and to estimate the size of the cells in each grid.

Control of environment pH with a CO_2 chamber

A hand-held cell culture in the atmosphere requires a CO_2 incubator to supply gaseous CO_2 to balance the bicarbonate buffering system, so as to maintain a culture medium in a physiological condition; cells require a partial pressure (5 %) of CO_2. In our work, CO_2 was generated in a fluid chamber filled with sodium bicarbonate that is simpler than the designs of Forry & Locascio,[12] and of Takano *et al.*;[13] we varied the concentration of solution to alter the pH of the cell-culture medium. We used the large permeability of PDMS for gases to diffuse CO_2 from the periphery of the chamber to the cell-culture area. The $NaHCO_3$ solution produces CO_2 gas; the concentration of $NaHCO_3$ influences the extent of CO_2 gas. With the diffusion of CO_2 and a varied amount of dissolution in the cell-culture medium, we measured the temporal variation of pH of the culture medium. As the amount of culture medium ($\fallingdotseq$ 21 μL) is tiny, we used highly precise pH test paper (Advantec No. 20, Grade, pH 5.0-8.0) to measure directly the pH at the open end. In this way, we decreased the error resulting from the content of CO_2 in the solution and the atmosphere balance.

We applied the formula of Takano *et al.*[13] to calculate the partial pressure of carbon dioxide. A $NaHCO_3$ solution (concentration 0.8 M) provides considerable partial pressure of CO_2 (5 %). In cell culture, using the principle of a communicating pipe, we continually provided aqueous $NaHCO_3$ solution at a fixed concentration (0.8 M) into the CO_2 chamber, the flow rate was controlled at 0.5 ml/min. The generated CO_2 gradually diffused into the culture zone, and dissolved in the cell-culture medium that was maintained in a steady state. The blue line in Fig. 4 indicates that, initially, the pH of the cell culture medium was 7.8; after 10 min the pH was about 7.7. The pH gradually decreased until the CO_2 of the external area and the internal area attained equilibrium. After 50 to 60 min, the concentration gradient of CO_2 no longer existed, and the pH of the culture medium was about 7.4. The purple line in Fig. 4 indicates that, when the peripheral aqueous sodium bicarbonate was removed, the dissolved concentration of CO_2 of the cell-culture medium exceeded that of the external environment; the CO_2 hence overflowed and increased the pH in the cell-culture medium in 1 h from the original 7.4 to 7.8. On removal of the external solution of sodium bicarbonate, we proved that we could vary the pH of the local environment of the nurturing cells. The pH-sensitive cells grown on the chitosan surface therefore readily and smoothly detach and dissolution.

Under continuous heating and no new perfusion, we tested the period to let the cell medium become completely dry. The CO_2 chamber filled with water (13 h, n = 12) prolonged 4 h more than that filled with air only (9 h, n = 12). This design hence also decreased the evaporation of the chip liquid. In addition, in this work we used the principle of a communicating pipe to provide continuously a fresh cell-culture medium into cell culture chamber to displace cellular waste and $NaHCO_3$ solution into CO_2 chamber to maintain CO_2 at the desired concentration during cell culture.

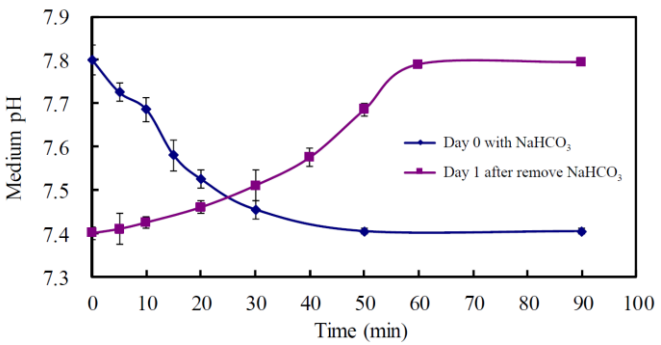

Figure 4. Temporal variation of medium pH in the culture area. When $NaHCO_3$ (0.8 M) flowed into the CO_2 chamber, the pH of the medium altered from 7.7 to 7.4 (blue line). After cells were cultured one day, we removed $NaHCO_3$ from the gas chamber. The pH of the medium spontaneously increased from 7.4 to 7.8 in 60 min (purple line). The pH in the culture area was measured with pH test paper.

Seeding, counting and scaling cells

The advantage of the MD-CaMP design is that, like a chamber slide, it can provide the same clarity of image vision. We used the grid of the micro-heater to count when seeding cells into the culture area and as a scale bar when observing the cells (Fig. 5). This technique of cell counting and seeding is unique in that all processes are completed in one step, so greatly abbreviating the experimental processes in the traditional way. In Fig. 5, when CL1-0 cells were seeded into the cell culture chamber with the grid of the micro-heater on the bottom, by designing the total bottom area of the cell culture chamber to be 21 mm^2 (including inlet and outlet zone) and the grid

area of the micro-heater to be 6.25×10^{-2} mm^2, we discovered the approximate amount of seeding cells to be 10,416, i.e., 336 grids in the micro-channel multiplied by 31 cells, the average number of cells in each grid. Also, the grid image on the bottom of the culture area can serve as a scale bar when observing the cells, as shown in Fig. 5. As the dimension of the grid is 250 μm × 250 μm, we calculate that the cell size of CL1-0 is about 20 μm.

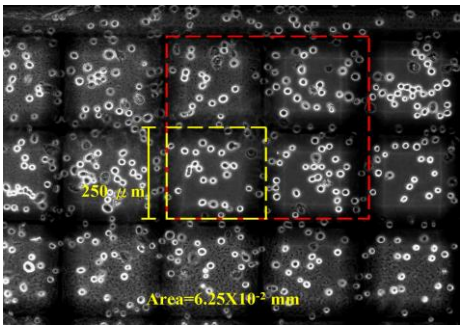

Figure 5. The image in the yellow region shows that CL1-0 cells were seeded in the culture micro-channel, above the micro-heater of grid form; the dimensions of the grid were 250 μm × 250 μm.

Microfluidic cell culture and fractionation can all be monitored

We developed a method to fractionate cells using a varied rate of detachment of cells to isolate cell fractions equated with biological indicators of the strength of the ability, such as the metastatic ability of cancer cells or the rates of oxygen consumption in cells (OCR) in vitro.[26, 27] In vivo, that cell detachment promotes tumorigenesis and leads to metabolic alterations such as decreased glucose uptake and decreased levels of ATP (adenosine triphosphate). The detachment of cells from the surface of the substrate is at present mainly provided with a conventional proteolytic enzyme treatment that might impair some cell functions and complicate the cell culture. We sought to use chitosan to replace protease so as to decrease the cell damage. Cells were therefore grown and detached from the chitosan surface on controlling the pH of the environment; protease-based solutions, such as trypsin, are no longer required.

Correlation of migratory ability with rate of detachment on pH-responsive chitosan

The complicate interactions between tumor cells and an extracellular matrix play important roles during tumor metastasis. Fibronectin plays a major role in oncogenic transformation, contributing to cancer cell growth and migration.[27] The degree of cell attachment on chitosan depends largely on the interaction between extracellular fibronectin and chitosan, which is sensitive to a variation of pH within the physiological range. When the environmental pH increased, the interaction between chitosan absorption and fibronectin decreased; more fibronectin-dependent cells tended to detach from the chitosan-coated surface more rapidly than other cell types.[19] To test the model, we chose the CL1 series of human lung adenocarcinoma cells, of which the CL1-5 subline is the most migratory in three human lung adenocarcinoma sublines whereas CL1-0 and CL1-1 show slower rates of migration as confirmed in our wound-closure assay. Figure 6(a) displays our experimental scheme to demonstrate the differential rates of detachment of CL1 sublines as the culture condition was altered from pH 7.4 to 7.8, under a simulated infusion of physiological medium (8 mm/s in artery = 64 mL/h flow rate in our chip) into the MD-CaMP. As clearly shown by the time lapse phase contrast images of the cells remaining in MD-CaMP in Fig. 6(b), we quantified the number of cells attached per unit area, in percentiles to the original number present in the same area at each given assessment point in Fig. 6(c). Notably, Fig. 6(d) shows that even the CL1-5 cells displayed substantial detachment from the chitosan surface only after pH changed to 7.8. Taken together, we conclude that the design of MD-CaMP and our operating scheme can efficiently discriminate cells with differential degree of metastatic ability.

Fractionation and analysis of live cells

To test the ability of a MD-CaMP to separate cells according to their metastatic ability, we co-cultured two cell lines, namely CL1-1 and CL1-5, together in the micro-channel to observe the cell-cell interaction on the chitosan surface. Figure 7(a) shows the experimental scheme of cell fractionation in the culture channel.

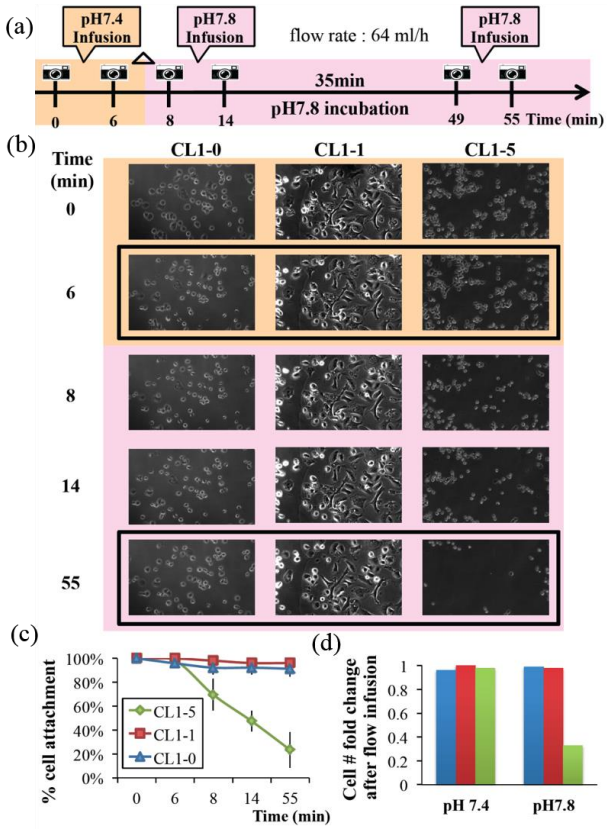

Figure 6. Differential cell attachment assessed by MD-CaMP. (a) Experimental scheme. (b) Time-lapse images of the cells in MD-CaMP upon infusion of buffered media that simulates physiological blood flow rate. **(c)** Quantitative representation of the percentage of cells that remained attached on chitosan surface at the indicated times. **(d)** The fold change of cell fractions attached while infusing with pH7.4 medium as compared to pH7.8 medium.

Figures 7(a,b) show the time-lapse images of co-cultured cells in a MD-CaMP of fluorescently labelled CL1-1 cells (red) and CL1-5 (green) cells upon introduction of a buffered culture medium at the indicated pH under infusion of physiological simulated blood flow through a micro-flow syringe pump. When cultured on a MD-CaMP, neither CL1-1 nor CL1-5 cells detached under simulated blood flow at pH 7.4, indicating a stable interaction between chitosan and cellular fibronectin. However, after switched to pH 7.8, the percentile of attached CL1-5 cells decreased sharply, from 78% to almost none at the end of the simulated infusion. In contrast, CL1-1

maintained about 90 % cells attached throughout the experiment as shown in Figure 6(c). These results clearly indicate that a sharp distinction of attachment behavior can be observed from each cell line with a time dependent fashion. We conclude that our chip design is capable of discriminating cells with different metastatic potential, even in mixed cell populations as the clinical specimen are. We believe that the MD-CaMP design can help fractionate biopsy or operative samples to provide a rapid index of the cancer metastatic potential.

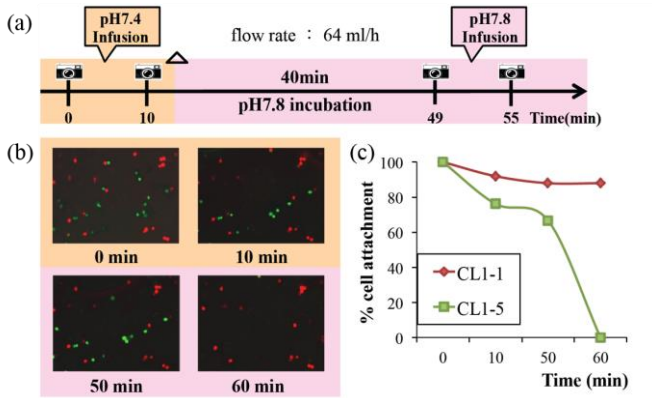

Figure 7. Selective fractionation of cells from a mixed population. (a) Experimental scheme**.** (b) Time-lapse images of fluorescently labeled CL1-1 cells (red) and CL1-5 (green) cells co-cultured in MD-CaMP. Buffered media at the indicated pH are infused through a micro syringe pump to simulate the physiological blood flow rate. (c) Quantitation of the cells remained attached to a same region of chitosan surface is recorded by fluorescence microscopy at the indicated time points.

Altered cell morphology with pH

To exclude the possibly that the raise in medium pH may cause cellular damages that lead to detachment from chitosan, we followed the change in cell morphology using DIC imaging (Figure 8), We found that CL1-5 cells were still completely attached to the surface after 60 min at pH 7.4 for a medium with chitosan-coating. When the pH was switched to 7.8, the cells gathered and began to detach.

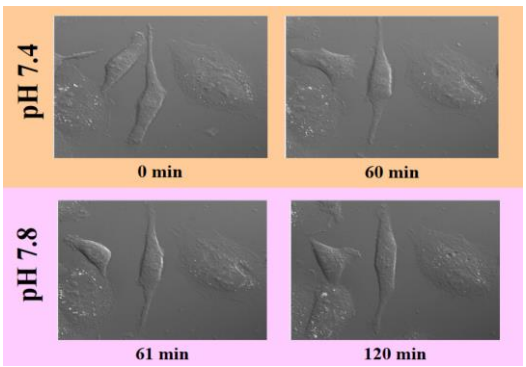

Figure 8. Morphological change of CL1-5 cells in MD-CaMP under incubation in different pH media as monitored by DIC microscopy.

Conclusions

With a major implementation, we proved that a MD-CaMP can fractionate cells to offer a rapid index of cancer metastatic potential. The features are that only a small specimen is required (e. g. from a biopsy), it is a portable device for diagnoses on site, temperature and CO_2 are controlled to maintain a basic growth environment, and the system functionally discriminates the migratory capability of cells. We expect that a MD-CaMP can provide valuable information for the planning of optimal treatment in a clinic. This design also offers an inexpensive nature, with both cell separation and observation of living cells in a miniaturized microfluidic incubator that allows both cell culture in the atmosphere and recording cell images with a conventional microscope. This component can serve for cell screening, fractionation of a cancer cell population and immediate observation of protein secretion. Our chip combines the advantages of both commercial closed- and perfusion-type microincubation image-chamber slides: little pollution and modifying the medium or addition of drugs are possible, providing high-resolution cellular imaging better than a plate or microwell. The device offers a uniform and stable distribution of temperature (37 ℃) in the area of cell culture. In addition to the grid-form micro-heater quickly adjusting to the appropriate temperature, the device can be used also for cell counting, trajectories of cell migration or as a scale bar when observing cells or DNA. We utilize $NaHCO_3$ solution through a gas-permeable material (PDMS) to give cells CO_2 at a partial pressure (5 %). At the same time, the humidity of the external environment is maintained and evaporation of the medium is slowed. Cells can thus grow and propagate in this device. Furthermore, we can control cell attachment to and detachment from the surface of pH-sensitive chitosan on varying the concentration of CO_2 through a permeable PDMS wall. With a varied rate of detachment of cells, we can effectively separate specific cells and obtain a pure cell type for downstream research.

The future potential of a MD-CaMP can include use in a clinic as a quick screening tool such that a biopsy specimen with violations of unknown cells of varied degree becomes separated with this chip. In research, co-culture of two or more cell lines can be studied through the cell-cell interaction and stimulation of cytokines, and then with purification with separation as a single cell line, ready for subsequent genomic or proteomic testing. We believe that this device will have a great benefit for the development of biological and pharmaceutical research such as gene expression, protein expression and functional assay.

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Notes and references

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