THE MINIUMUM TIME ESTIMATION FOR INITIATING TUMOR-CELL ATTACHMENT

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ABSTRACT

This work presents the first part of a new framework for preventing the tumor-cell of carcinoma in situ transition from one organ to others. Using an ECIS (electric cell-substrate impedance sensing) chip coated with glutaraldehyde (GA)-crosslinked gelatin patterns suitable for cell attachment, the authors monitor the cell adhesion situation not only by optical microscope but also by electrical means. Therefore the authors design an experiment and a microfluidic chip for investigating the relationship between the metastasis and the surface morphology of blood vessels.

KEYWORDS

ECIS, cell attachment, metastasis, liver cancer

INTRODUCTION

A 1 mm-size carcinoma in situ releases millions of tumor-cells per day [1]. Via blood circulation in Fig. 1, most of those tumor cells squeeze and pass the capillary network [2], but some of them are stuck (in another organ) and begin growing. Other than the conventional gene expression interruption for preventing the tumor transition, a new physical methodology of formulating surface morphology of the inner walls of capillaries so hard as to attach cells is now under investigation.

If this anti-attachment methodology is feasible, all the released tumor-cells would be hard to transfer from organs to others. To realize the above idea, two issues should be confirmed: (1) Which kind of tumor-cells is critically needed to be investigated accordingly? (2) What detailed experiments of investigation should be done? The authors tried to discuss the first issue in this introduction section and selected liver cancer as the research target.

Compared to the normal blood circulation schematic of Fig. 1(a), the liver has an unusual blood supply system with an input entrance of veins (portal vein) shown in Fig. 2 [3]. On the one hand, like other organs, the liver receives blood containing oxygen from the heart. This blood enters the liver through the hepatic artery, accounting for one quarter of the liver. On the other hand, liver receives blood filled with nutrients, or digested food particles, from the small intestine. This blood enters the liver through the portal vein, accounting for the majority (three quarters) of the liver. In the liver, the hepatic artery and the portal vein branch together into a capillary network of tiny blood vessels. This manner is quite different from most of organs with only portal artery.

Cancer from other parts of the body often spread to liver. The process of metastasis involves an intricate interplay between altered cell adhesion, survival, proteolysis, migration, lymph-/angiogenesis, immune escape mechanisms, and homing on target organs [4]. Herein, the authors left alone the most of the complicated metastasis mechanism of liver cancer but focused on the anti-attachment of tumor-cells in a mechanical manner. The blood pressure near the portal vein of liver is believed to be lower than the input entrance of other organs and is more apt to block cells in the corresponding blood vessels.

Some pathology information about cirrhosis is provided as below: Liver is the strongest organ of regeneration capacity. In general, the normal liver as long as reservation about 20 to 30 percent of the volume for living is enough, and the remaining liver tissue can re-growth to near its original size in 3 to 6 months. However, if the portion near the portal vein has been invaded by cancer or cirrhosis [5], two most threatening diseases of liver, it's unsuitable for surgery.

Cirrhosis is a disease of the liver in which scar tissue forms throughout the organ. Regenerative nodules, surrounded by sheets of scar tissue, replace the normal spongy tissue of the liver and cease some vital functions. The affected tissue may keep blocking the blood flow, causing high pressure in the liver blood vessels. Internal bleeding may then result in. In addition, the blockage may lead to the accumulation of fluids inside the abdomen.

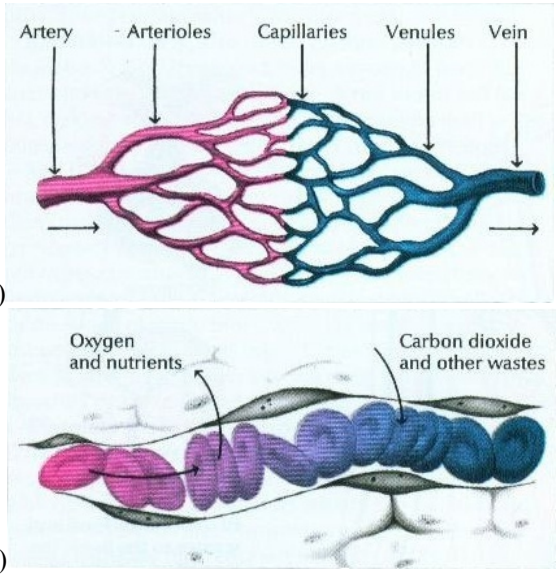

Figure 1: Prevention of tumor-cell attachment in blood circulation: (a) Capillary exchange system for the blood; (b) Most capillaries are so small that blood cells as well as tumor cells must pass them in single file [2].

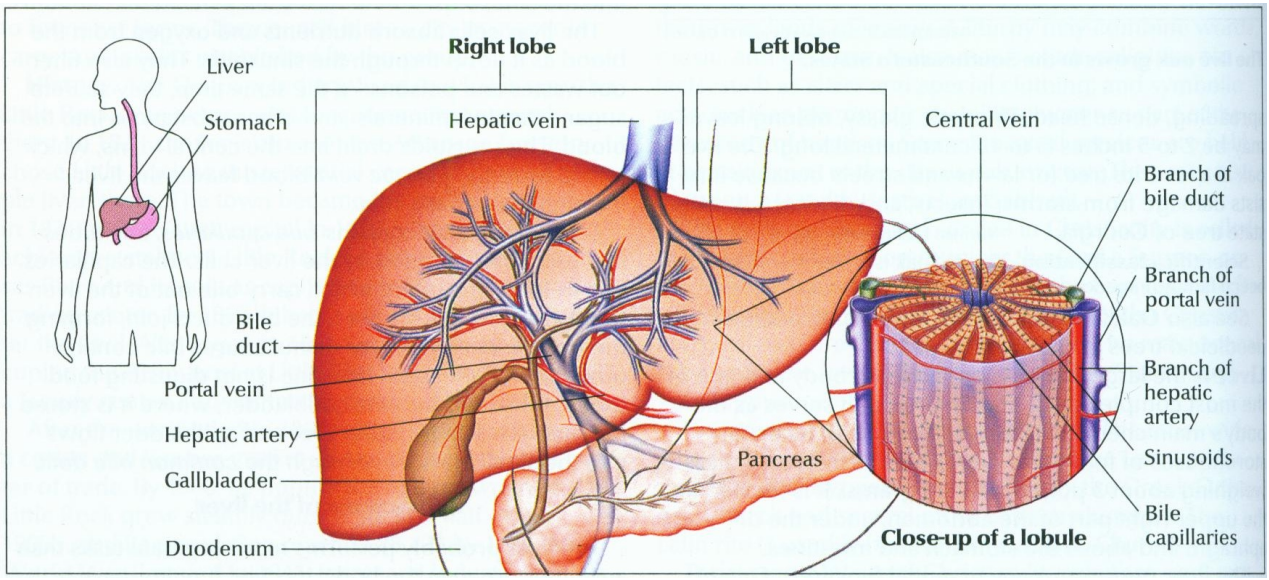

Figure 2: The liver structure [3].

Restated, the second issue in this paper is to design an experiment for investigating the anti-attachment behavior of liver cancer cells, HepG2. The proper time duration for doing microfluidic experiments related to the prevention of the tumor-cell transition is the first information necessary to the whole framework.

MATERIALS AND METHODS

The authors designed an experiment setup with a microchannel chip for investigating the interaction between the surface morphology and the tumor-cell attachment. Figure 3 shows this experiment design. The cultured HepG2 living cells are delivered from a reservoir to a microchannel chip which animates the capillary blood vessels. The attachment behavior of HepG2 to the capillary microchannel is optically monitoring by a bright-field inverted microscope during the flow pumping which animates the blood circulation.

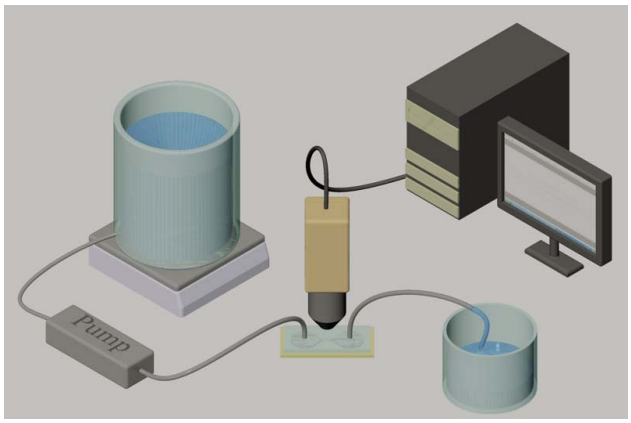

Figure 3: Schematic diagram of experimental design.

The microchannel chip fabricated by soft lithography is composed of a glass substrate with a PDMS channel cap in Fig. 4. The observation area is with 500 μm long, and the hydraulic diameter of microchannel bottleneck is designed as 12-23 μm, comparable to the dimension of capillaries. The micro- environment of the microchannel chip and the cell reservoir should have the macro culture condition of 37°C and CO_2 rich.

Direct manipulation of the experiment in Fig. 3 without proper control conditions may result in meaningless result of tumor-cell anti-attachment. If we regard Fig. 3 as the dynamic case of cell attaching experiment, a static case of cell attachment without moving flow (the general cell culture) is accordingly supposed to be done in advance. Additionally, some specific microsensors are necessary to integrate into the microchannel chip for the reason of sensing redundancy.

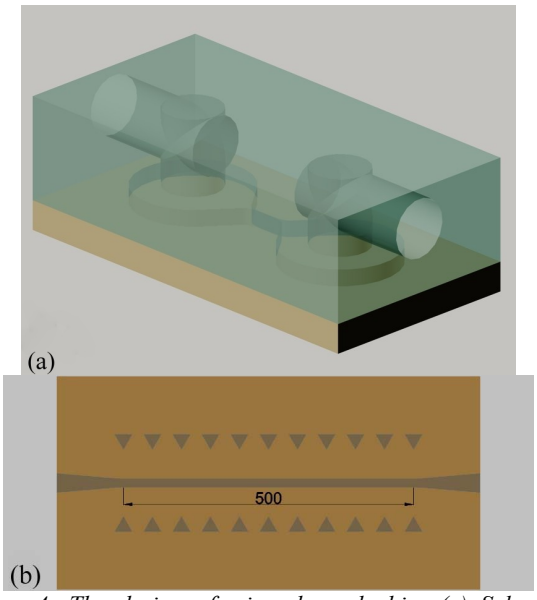

Figure 4: The design of microchannel chip: (a) Schematic diagram of 3D model and (b) Observation area (unit: μm).

The optical microscope observation can't be solely

used in monitoring cell growth at the initial stage of cell adhesion to the gelatin substrate. It is somewhat hard to judge whether the cell adhesion is good or not. Additional confirmation is to detect the impedance by ECIS chips herein [6-7]. As cells adhering on electrodes will lead to the impedance increasing. The authors also coated the ECIS chips with glutaraldehyde-crosslinked gelatin patterns suitable for cell attachment [8].

In conventional manner, 3-day cell culture with a day-interval observation is often used. We wonder the time resolution of 1-day is not enough for tumor-cell adhesion and would like to have a better time resolution of 1-hour confirmed by ECIS chips [9]. The ECIS chip is designed as Fig. 5(a) and made of transparent ITO electrodes. A parylene film is covered and patterned above the ITO as the insulator layer. Finally gelatin micropatterns spread over the ITO electrodes as the role animating the laminin protein or extra cellular matrix of capillaries for attracting cells [8, 10-12] as Fig. 5(b). The processing of ITO electrodes and gelatin patterns are shown in Figs. 6 and 7, respectively [13].

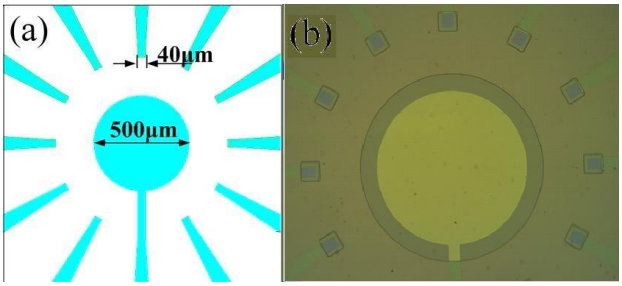

Figure 5: Schematic diagram of the ITO electrodes: (a) The close view of the core region. The counter electrode with 500 μm diameter and the working electrodes with 40 μm tips; (b) Gelatin micropatterns fabricated on the working electrodes.

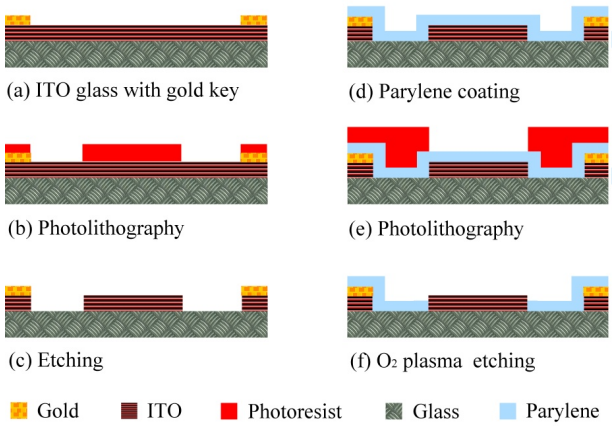

Figure 6: Illustration of the fabrication process for the ITO electrodes.

Figure 8 shows images of HepG2 cells adhesion on the electrode and Fig. 9 shows the frequency response of the chip impedance. The electrode and the cell are similar to an in-series resistor connection. The magnitude of the impedance relates to the adhesive area of cells. That means, we can predict the cell adhesive condition from the output impedance profile.

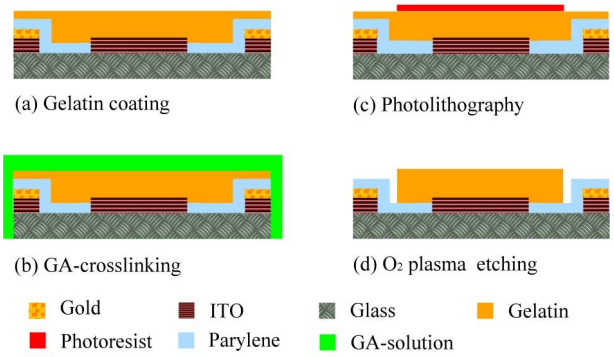

Figure 7: Patterning process for GA-crosslinked gelatin: (a) Spin coating of pure gelatin film; (b) Crosslinking of gelatin film in 50% GA solution; (c) Patterning of positively toned photoresist mask on the gelatin film; (d) Etching with O_2 plasma and stripping of photoresist with acetone.

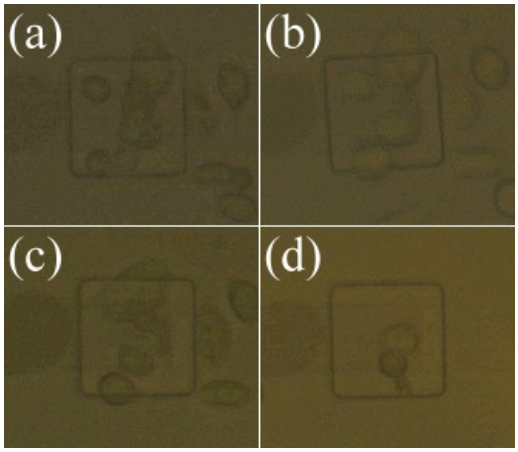

Figure 8: Cell images under the inverted microscope: (a) Seeding after 4 hours (curve-0550 in Fig. 9); (b) 5 hours (curve-0650); (c) 6 hours (curve-0750); (d) 7 hours (curve-0850).

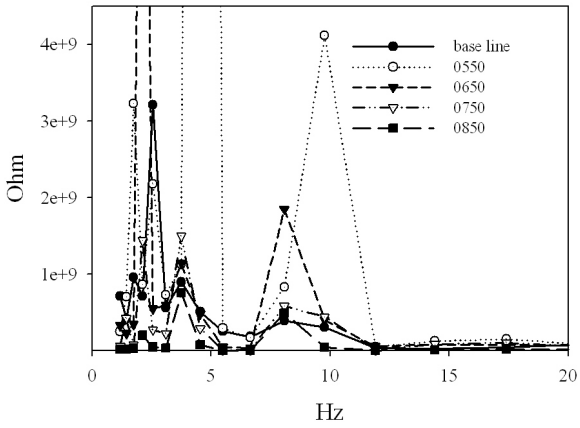

Figure 9: The electrode signal of cell adhesion; baseline denoting no cell adhesion on the electrode.

Not all of the eleven electrodes in Fig. 5(b) successfully attach cells; these electrodes with cell-attachment may malfunction during the cell culture. The living situation of the attached cells should be

identified by the inverted microscope of Fig. 8 before every ECIS measurement. The maximum impedance in Fig. 9 shows that the cell attachment area reaches its maximum value for 4 hours (curve-0550) after the cell dosing. After 4 hours, the cell-attachment density, the impedance response as well as the attachment area all decrease with time. After 7-hour culture, the impedance response approaches back to the initial case or even short circuited. This cell-culture experiment with 1-hour time resolution so far demonstrates that the attachment moment for HepG2 on gelatin surface is no longer than 4 hours after the cell dosing and these tumor cells cannot stay on GA-crosslinked gelatin surface for more than 7 hours.

In summary, the estimation of the minimum time for tumor-cell HepG2 attaching on gelatin surface is no longer than 4 hours after the cell dosing. This information is the first guideline for preparing the sufficient amount of dosing tumor-cells in the fore coming dynamic experiment of Fig. 3.

CONCLUSIONS

The authors used an ECIS chip to evaluate the cell adhesive situation from the output impedance profile. As the adhesion area is raised, the impedance increases. The ECIS chip herein integrates GA-crosslinked gelatin micropatterns which are suitable for cell attachment. The estimation of the minimum time for tumor-cell HepG2 attaching on gelatin surface is no longer than 4 hours after the cell dosing. The dynamic flowing test of HepG2 cells in a PDMS microchannel is looking forward as the next step of the anti-attachment of tumor-cell framework.

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