# Dynamic Cell Attachment of HepG2 in a Microchannel

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*Abstract*—**A dynamic filling experiment using PBS with HepG2 tumor cells is performed for observing the attachment behavior in capillaries. A PDMS microchannel mimicking the capillary blood vessel is fabricated by the soft lithography. With treating the PDMS microchannels by different plasmas, the corresponding surface roughness ($R_a$) data are experimentally measured by AFM. Using these PDMS microchannels well defined by proper $R_a$ values, liquid streams with microbeads and living HepG2 tumor cells are filled in, respectively. The microbead and cell attachment areas in the PDMS microchannel have been recorded dynamically with 10 min interval. Finally, the authors qualitatively discussed the surface roughness effect on the particle or cell attachment in a PDMS microchannel.**

***Keywords-microchannel; blood vessels; fractal; PDMS***

## I. 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 like most blood cells. But these few tumor cells may be stuck in another organ and begin its growing over there. 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, homing on target organs, and so on [2]. Herein, the authors left alone the most of the complicated metastasis mechanisms of liver cancer but rather focused on the anti-attachment of tumor-cells from a mechanical perspective.

Compared to the normal blood circulation in Fig. 1, the liver has an unusual blood supply system with an input entrance of the portal vein [3]. The blood pressure near the portal vein of liver is believed to be lower than the pressure at the entrance artery of other organs and may be more apt to block tumor cells in the corresponding blood vessels [4]. In this paper, the authors therefore design an experimental framework for investigating the anti-attachment behavior of HepG2 cells and consider a flow in a single channel made of PDMS as Fig. 2. It tries to especially animate the actual blood flow case through the liver portal vein subject to serious clogging of tumor cells or scars tissues.

By the conventional (static) cell culture, tumor cells have found to attach selectively on certain surfaces with, e.g., gelatin [5] or something like that. From a mechanical point of view, rather than regarding the surface science or biological aspects on this cell attachment issue, the authors intuitively wonder if the surface roughness may have any substantial influence on the attachment behavior of tumor cells.

Apparently under the laminar situation of low Reynolds number different from the turbulent case, the pure liquid flow pattern seems to have no matter with the surface roughness of the channel inner wall [6]. (In other words, any disturbance induced by surface roughness will be eliminated in the laminar region.) However, for the liquid flow with particles like cells herein, the previous argument prevails no more. So the authors conducted a dynamic filling experiment on the micro channels with different surface roughness as follows.

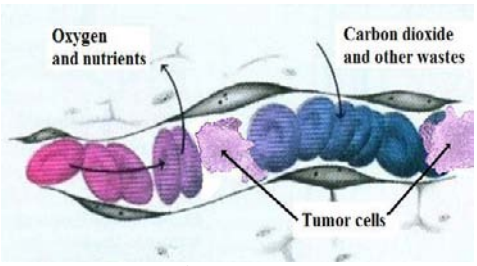


Fig. 1. Prevention of tumor-cell attachment in blood.

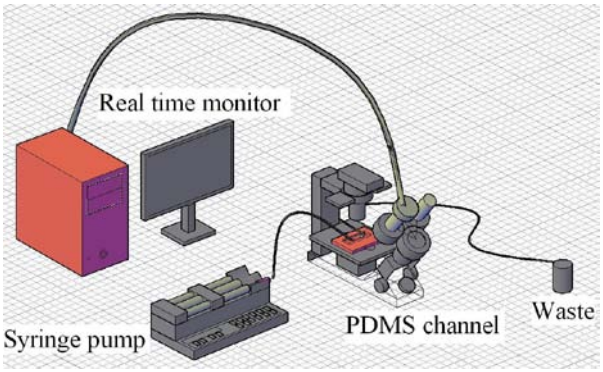


Fig. 2. The experimental setup of animating the dynamic filling into a circulation, especially at the port vein of liver.

## II. FABRICATION AND EXPERIMENT

### A. *Fabrication of a PDMS Microchannel*

The single channel of the PDMS flow chip in Fig. 3 [4] is with 500 μm long, 15 μm wide and 5 μm high. The hydraulic diameter of the microchannel bottleneck is designed as 7.5 μm comparable to the dimension of capillaries. The fabrication process of the popular PDMS soft lithography using SU-8 resist mold is depicted in Fig. 4. It is composed of a glass substrate with a PDMS channel cap.

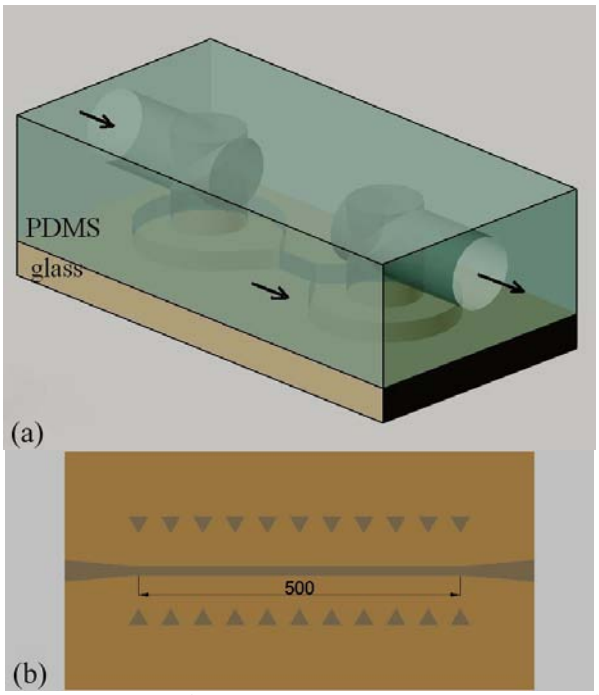


Fig. 3. (a) 3D model of the single-channel chip; (b) the portion of the observation area (unit: μm) [4].

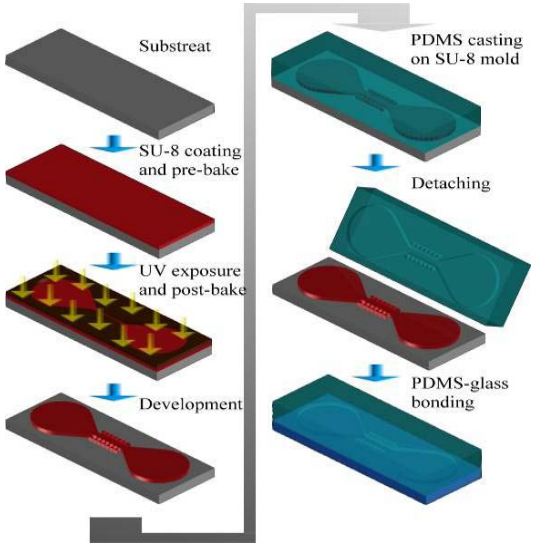


Fig. 4. Frabrication of PDMS michannel.

### B. *The $R_a$ Measurement of the Microchannel Wall*

Before the dynamic filling experiment, the surface roughness data of the inner bottom of all the PDMS microchannels are verified by AFM and summaries in Fig. 5. The PDMS channels have been etched by plasma ($CF_4$: $O_2$=1:1) from 3 min to 15 min with different RF powers. The maximum $R_a$ which the plasma can achieve is about 20 nm herein. The PDMS chips still can be firmly bonded to glass slides as closed channels under such plasma etching condition.

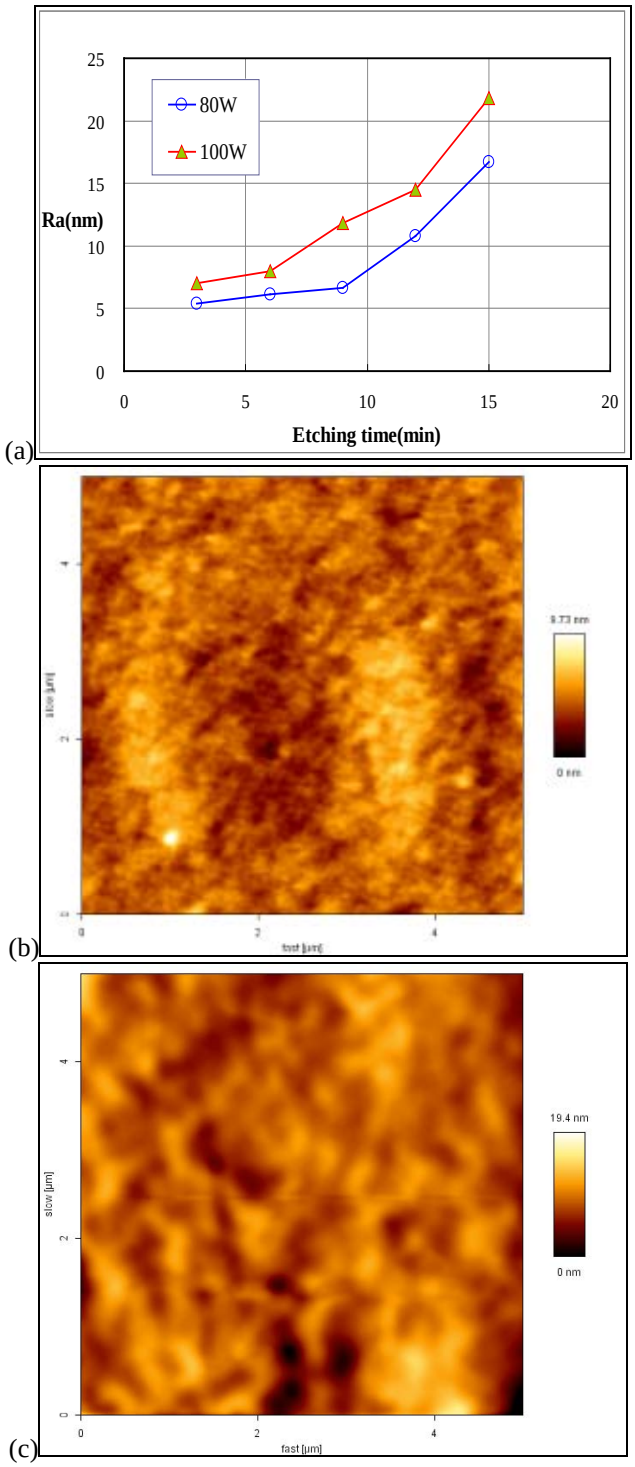


Fig. 5. Surface roughness ($R_a$) of a PDMS microchannel (inner bottom) subject to different plasma etching conditions ($CF_4$: $O_2$=1:1; plasma etching for 15 min): (a) $R_a$ data; (b) an AFM figure before etching; (c) an AFM figure after 15-min, 100W etching.

### C. *Dosing Concentration of HepG2 Cells*

As people know, there is about 5 liter of blood for an adult human. The authors neglect the concentration variation of tumor cells in man's body and assume no metabolism of these tumor cells in the blood circulation system. The maximum cell concentration in blood after 1 day of 1 million tumor cells releasing is easily calculated as

$$10^6\, cells \div 5L = 2\times 10^5 \frac{cells}{L} = 200\frac{cells}{mL} \qquad (1)$$

The general cell concentration during cell culture often surpasses the value of (1) very much. After several times of tries, the authors found that the 5 orders of magnitude larger concentration about $1.1\times10^7$ cell/mL is a more appropriate value to the cell concentration for the dynamic filling in this work.

### D. *Flowing Conditions in the Single Microchannel*

The flowing conditions are mentioned in this section. The red blood cell (RBC) velocity in ordinary capillaries is believed to be 6.8 μm/s [7]. However, there's too small amount of tumor cells (with the number of about order 1 or even less) entering into the PDMS microchannel in 2 hours under such a slow velocity to be observed. The reason for not performing the 72-h dynamic filling is that so far the authors still have no proper facility to ensure the good culture condition of 37°C and $CO_2$ rich for the experiment setup of Fig. 2.

Referring to the dosing concentration of living cells in the previous section, the authors also found a more feasible filling velocity in capillaries herein should be 5 orders of magnitude faster than the ordinary case. For example, if the PBS filling velocity is increased from the 6.8 μm/s dramatically to 3.77 m/s or with the equivalent volumetric flow rate of 10 μL/min (crossing a 7.5 μm-diameter microchannel), then it may pump $1.32\times10^7$ tumor cells into the single channel for cell attachment in 2 hours. Of course, such a high flow rate induced by high pressure which can be predicted by the Hagen-Poiseuille law [8] (not shown here) is not the ordinary case of capillary blood flow, but rather like the flow case through the liver portal vein subject to serious clogging of tumor cells or scars tissues.

### E. *Cell and Microbeadss Filling Test*

The cultured HepG2 living cells were delivered from the cell reservoir to a single channel PDMS chip in Fig. 3. The attachment behavior of HepG2 to the capillary microchannel was optically monitoring by a bright-field inverted microscope during the flow pumping. The authors additionally performed the filling experiment using polystyrene (PS) microbeads of 5 μm size as the comparison counterpart. Table I summaries the attachment modes of microbeads and HepG2 cells after the 2-h filling with a flow rate of 10 μL/min.

From Table I, the PS microbeads with a regular sphere shape like RBCs behave neither particle attachment nor blocking phenomena during the dynamic filling experiment, no matter how the surface roughness of the PDMS channel is. On the other hand, after attach-and-detach process HepG2 cells with more roughened surface and more complicated morphology like platelets or white blood cells (WBCs) behave more sticking manner. They attach and detach over and over again at the throat portion of the un-etched microchannel with $R_a$ of 3 nm. HepG2 cells moreover block the throat of the microchannel with $R_a$ of 20 nm in this 2-h filling.

Based on the observation of the clogging case (d) in Table I, the authors recorded the attachment area change of HepG2 cells in a PDMS microchannel for every 10 min corresponding to different etching conditions in Fig. 6. Restated, the values of $R_a$ for the etched and un-etched surfaces are 20 nm and 3 nm. The (etched) microchannel with larger surface roughness accumulates and attracts more tumor cells than the (un-etched) smoother microchannel, and is more apt to choke the PBS filling flow. For low Reynolds number flow (*Re*~O(1) herein), surface roughness conventionally should have no influence on the flow pattern and shear stress [6]. However, surface roughness did have substantial effect on the tumor cell attachment herein. The variation of the cell attachment area in Fig. 6 also reveals the strange segregation behavior of the suspension flow with micro particles [9-10].

Referred to [9-10], the authors suspected that PS beads like RBCs may move along the central line of the microchannel and has less chance to interact with the wall as well as the surface roughness; whereas HepG2 cells like platelets or WBCs were suspected to loiter near to wall and had more possibilities to stick to the wall or consequently correlated with the surface roughness. Whether the PS microbeads and the HepG2 tumor cells can be respectively suspected as RBC and WBC according to their flowing characteristics still need more evidences of dynamic filling experiments with respect to different flow properties, conditions, and may be left as a future work.

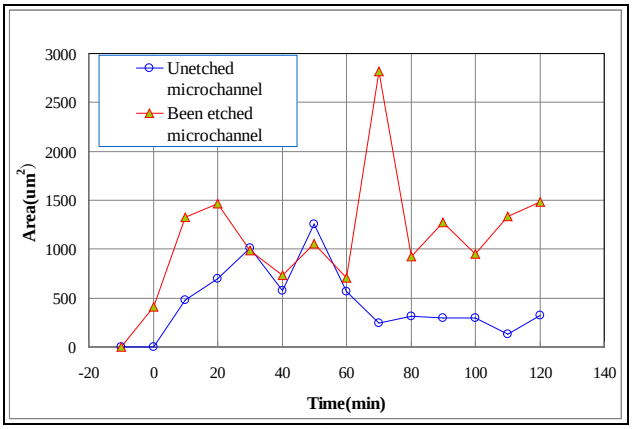


Fig. 6. Time-varying attachment area of HepG2 cells in PDMS microchannel corresponding to different $R_a$ (flow rate: 10 μL/min; cells diluted in PBS.)

## III. CONCLUSION

The final goal of this cell dynamic filling experiment is to figure out the largest surface roughness of a PDMS microchannel wall for ensuring the anti-attachment of HepG2 tumor cells. After several times of trying, the authors found a meaningful case manifesting the surface roughness effect with

the following conditions. Subject to the filling flow rate of 10 μL/min through a channel with hydraulic diameter of 7.5 μm and the cell concentration of $1.1\times10^7$ cell/mL, no cell blocking has been observed if the $R_a$ value is 3 nm (pure PDMS). A plasma-roughened PDMS microchannel with $R_a$ of 20 nm could not provide the sufficient anti-attachment capability for the HepG2 cells on the PDMS wall surface. More evidences of the similar PDMS microchannels with $R_a$ ranging below 100 nm but with different flow properties and conditions are also expected to be searched for advanced investigation.

## ACKNOWLEDGMENT

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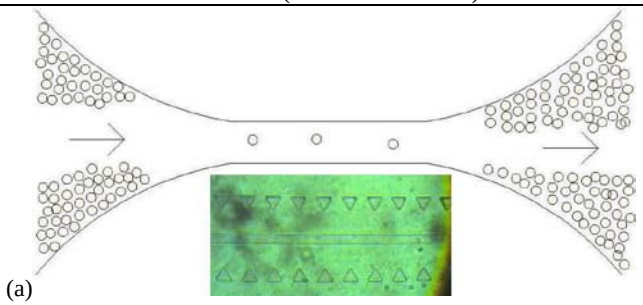
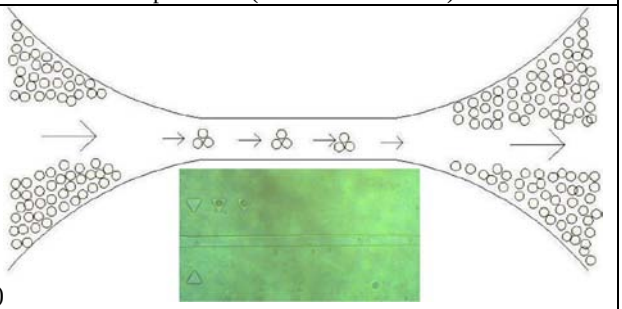
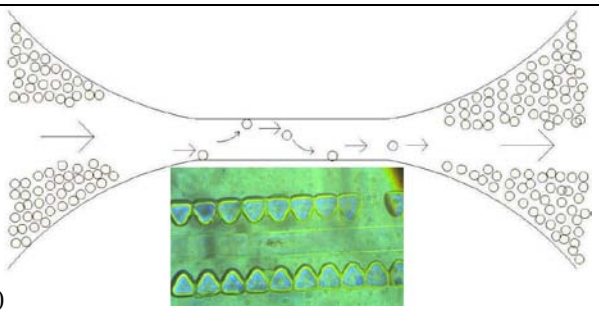
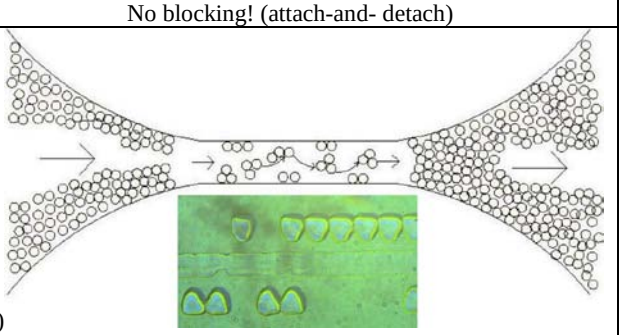
TABLE I. THE ATTACHMENT MODES OF MICROBEADS AND HEPG2 CELLS AFTER 2-HOUR DYNAMIC FILLING:

| | Microbeads (RBC-like behavior) | HepG2 cells (WBC-like behavior) |
|---|---|---|
| Small $R_a$ (3 nm), without plasma etching |  (a) No blocking! |  (c) No blocking! (attach-and- detach) |
| Large $R_a$ (20 nm), with plasma etching |  (b) No blocking! |  (d) Almost blocking! |