

# Dynamic Attachment of HepG2 in Fractal Microchannels

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**Abstract**—Dynamic filling experiments using HepG2 tumor cells are performed for observing the attachment behavior novelly in fractal microchannels. A PDMS microchannel mimicking the capillary blood vessel with fractal configuration is fabricated by the gelatin salt-out technique and the PDMS soft lithography. The fabrication of this low-toxicity fractal PDMS microchannel is firstly addressed herein. Using this PDMS microchannel, PBS with living HepG2 tumor cells are filled through. The cell attachment areas in the PDMS microchannel have been recorded dynamically with 10 min interval continuously for 2 hours. The result show that 2 hour is not enough for the permanent cell attachment of HepG2 in this fractal PDMS microchannel.

**Keywords**-HepG2; blood vessels; fractal; PDMS

## I. INTRODUCTION

Dynamic filling experiments of Fig. 1(a) using HepG2 tumor cells and polystyrene (PS) microbeads have ever performed for observing the particle attachment behavior in a PDMS microchannel [1]. 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. This work is a part of the authors' framework to control the particle adhesion in capillaries so as to interrupt the mechanism of tumor metastasis [2], gouty arthritis, and so on. The 1<sup>st</sup> step work or this dynamic filling experiment reported in NEMS 2013 [1] is to study the surface roughness effect on the HepG2 tumor cell attachment on a PDMS single channel from a mechanical point of view.

By the conventional (static) cell culture, tumor cells have found to attach selectively on certain surfaces with, e.g., gelatin [3]. This microchannel is subject to a dynamic filling of PBS dosed with living HepG2 cells. The polystyrene microbeads neither behave particle attachment nor block the microchannel during the dynamic filling, no matter how large the surface roughness of the microchannel is. HepG2 cells attach and detach over and over again at the throat of a single microchannel with 3nm roughness. HepG2 cells even block the microchannel throat with 20nm roughness after 2-hour filling [1]. This surface roughness effect on the cell attachment is quite different from the behavior of the general low-Reynolds number flow where the flow pattern has no matter with the surface roughness of the channel inner wall [4]. (In

other words, any disturbance induced by surface roughness will be eliminated in the laminar region.)

The drawback of this prior work [1] is that the PDMS microchannel is ideally designed with a rectangular cross section and with the hydraulic diameter of 7.5 $\mu$ m comparable to the capillary dimension. For the sake of additional investigating the effect from the microchannel geometry moreover, we novelly replace the single microchannel with a fractal microchannel [5] as Fig. 1(b) shows in this paper.

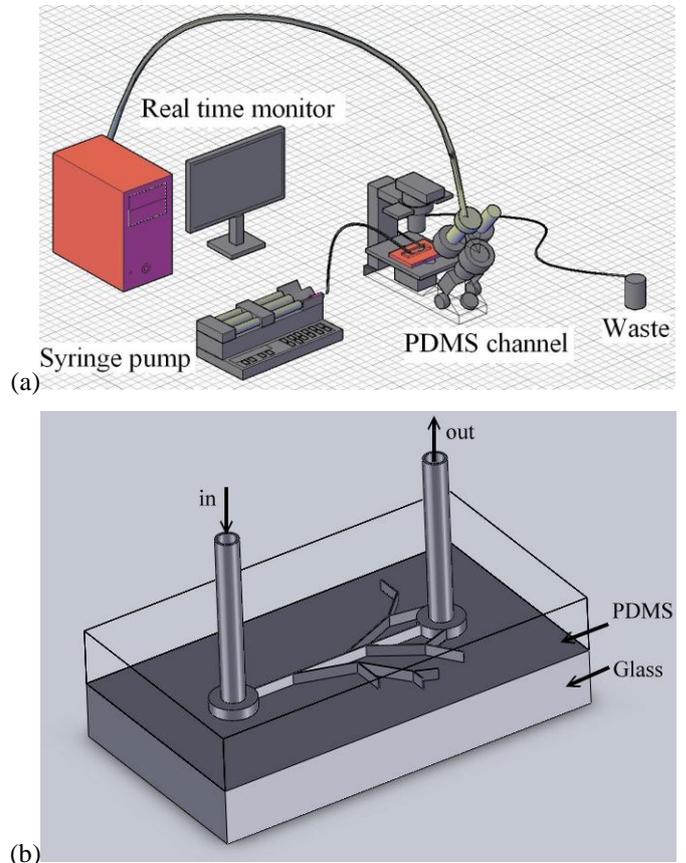


Fig. 1. (a)The experimental setup of animating the dynamic filling into a circulation, especially at the port vein of liver [1]; (b) the PDMS microchannel chip using in this paper.

## II. FABRICATION AND EXPERIMENT

Two parts of work at least have been done in this paper:

### A. Fabrication of low-toxicity fractal PDMS Microchannels

The original single microchannel of the PDMS flow chip in [1] is with the dimension of 500  $\mu\text{m}$  long, 15  $\mu\text{m}$  wide and 5  $\mu\text{m}$  high. So the hydraulic diameter of the microchannel bottleneck is designed as 7.5  $\mu\text{m}$  comparable to the dimension of capillaries. It is composed of a glass substrate with a PDMS channel cap. But in this paper we need a PDMS cap with fractal channel patterns. We resort this task to using the salt-out technique of over-saturated gelatin [5].

Fractal PDMS blood vessels have been reported using the salting process of over-saturated  $\text{K}_2\text{Cr}_2\text{O}_7$ -gelatin solution [5]. But this prior art has the toxicity problem. Living cells are hard to culture on it. Herein we newly developed another recipe of  $\text{K}_2\text{SO}_4$ -gelatin solution in this paper to reduce the toxicity of the fractal patterns. With this new recipe, not only the toxicity problem is not serious, but also the salting-out gelatin patterns are so obvious to be assigned as the convex molds for making PDMS fractal channels.

The detailed processing parameters have been fully investigated. One example of the 20wt% gelatin solution with different spin coating speeds corresponding to different concentration of  $\text{K}_2\text{SO}_4$  is shown in Fig. 2. The maximum height of the fractal pattern so far can be up to 9.2 $\mu\text{m}$ .

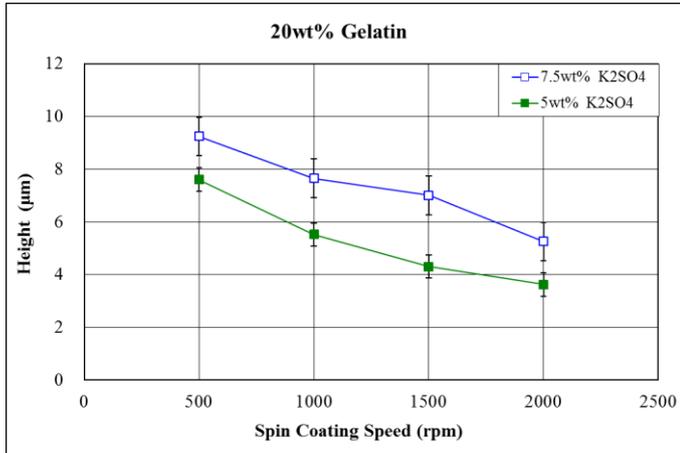


Fig. 2. The fractal height variation of the 20wt% gelatin solution with different spin coating speeds corresponding to different concentration of  $\text{K}_2\text{SO}_4$

We have ever used these salting-out gelatin films as the bases for culturing HepG2 cells on them to preliminarily demonstrate the toxicity of the  $\text{K}_2\text{SO}_4$ -gelatin. The cell density results after 3-day culture of Fig. 3 show the much lower toxicity of the  $\text{K}_2\text{SO}_4$ -gelatin fractal patterns compared with the  $\text{K}_2\text{Cr}_2\text{O}_7$ -gelatin. (There is almost no cell survived on the  $\text{K}_2\text{Cr}_2\text{O}_7$ -gelatin after 1-day culture; the cell density on the  $\text{K}_2\text{SO}_4$ -gelatin keeps stable for at least 3 days.)

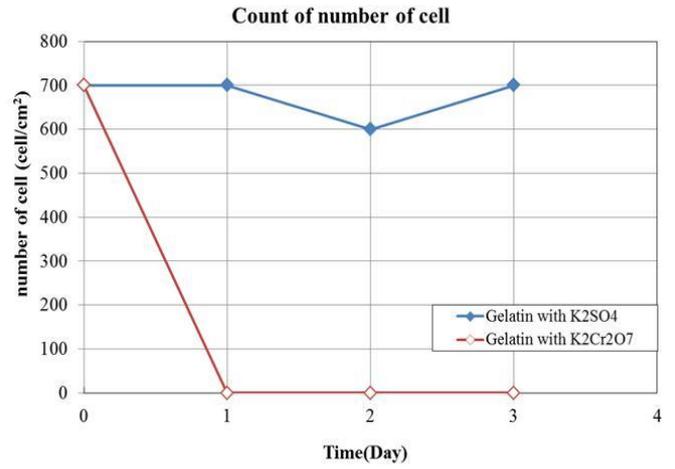


Fig. 3. HepG2 cell density vs. time for 3-day culture. The higher cell density denotes the lower toxicity

The fractal PDMS microchannels mimicking capillary blood vessels are fabricated by the mold transferring of the popular soft lithography with the convex motherboard of the low-toxicity fractal gelatin shown in Fig. 4.

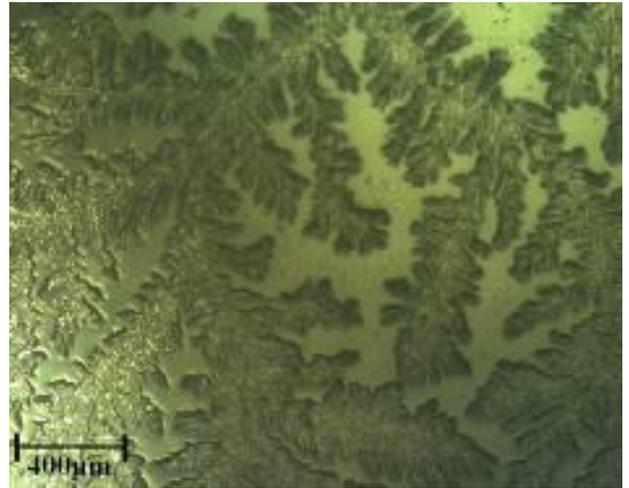


Fig. 4. Fractal gelatin pattern with the maximum height of 9.2 $\mu\text{m}$ ; 20% Gelatin-7.5%  $\text{K}_2\text{SO}_4$ ; 500rpm spin coating.

### B. The Dynamic Filling of HepG2 Cells in the Fractal Microchanne

#### 1) Dosing concentration of HepG2 cells

The proper cell dosing concentration during cell culture has been tried for many times. The authors finally found that  $10^7$  cell/mL is fine for the dynamic filling and cell attachment in this framework [1].

#### 2) Flowing conditions in the fractal microchannel

The flowing conditions are similar to what has been mentioned in [1]. 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 to up to 3.77 m/s or with the equivalent volumetric flow rate of 10  $\mu\text{L}/\text{min}$  (crossing an assumed 7.5  $\mu\text{m}$ -diameter

microchannel), then it may pump  $1.32 \times 10^7$  tumor cells into the single channel for cell attachment in 2 hours. The reason for doing only 2h dynamic filling is just to make sure the cells are living. (All cells are not preserved in the cultured vessel.) This flow case is to animate the liver portal vein subject to serious clogging of tumor cells or scars tissues.

### 3) Dynamic cell filling test

The cultured HepG2 living cells were delivered from the cell reservoir (the syringe pump) to the PDMS chip with fractal channels in Fig. 1. The attachment behavior of HepG2 to the capillary microchannel was optically monitoring by a bright-field inverted microscope during the flow pumping as Fig. 5(a-c). The filling times are 10 min, 60 min, and 120 min respectively. The attachment areas of the HepG2 cells in the PDMS microchannel are therefore recorded dynamically with 10 min interval in Fig. 6 and analyzed by the software ImageJ. Finally, we qualitatively discuss the fractal pattern effect on the cell attachment in PDMS microchannels.

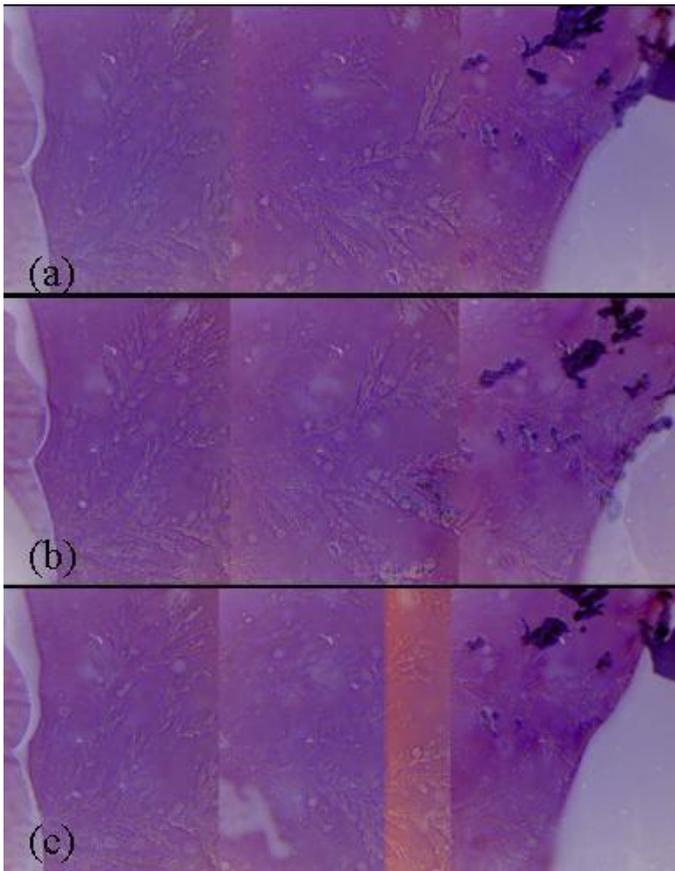


Fig. 5. HepG2 dynamic filling and attachment with 10  $\mu\text{L}/\text{min}$  along the PDMS fractal microchannels (left is entrance and the right is exit): (a) 10 min; (b) 60 min; (c) 120 min..

The prior dynamic filling experimental result of Fig. 6(a) shows that the cell attachment areas reach their saturation after 30min filling in a single, straight PDMS microchannel [1]. Even with the un-etched PDMS microchannel and the surface roughness of about 3nm, the saturated attachment area after 2h

is about  $300\text{-}400\mu\text{m}^2$ . The cell segregation phenomena may play important role in this experiment [6-8].

In this paper, the dynamic cell attachment area in the fractal microchannels with the similar hydraulic diameter and the surface roughness also increases with time, as shown in Fig. 6(b). But it has not yet reached its saturation situation in 2h. The maximum cell attachment area in a fractal channel is  $33\mu\text{m}^2$  and only one order of magnitude lower than the single channel case. The preliminary reason may be that the longer loitering range or the larger holding capacity provided by the chaotic microchannel for cells herein needs longer time to implement the cell attachment process as well. It needs more experimental data to justify this argument.

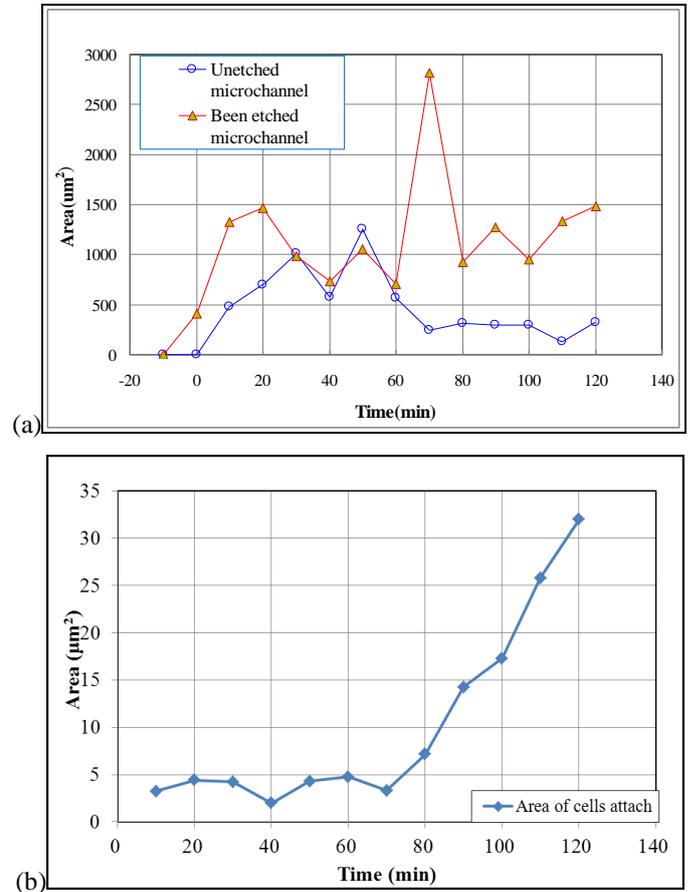


Fig. 6. HepG2 dynamic attachment area change vs. time for 2 h: (a) single microchannel [1]; (b) fractal microchannels.

### III. CONCLUSION

In this paper, we fabricated the low-toxicity fractal PDMS microchannel by the salting-out method using over-saturated 7.5%  $\text{K}_2\text{SO}_4$ -20% gelatin solution. The largest height of the fractal pattern is  $9.2\mu\text{m}$ . Secondly we performed the dynamic filling experiment on this new flow chip with a fractal microchannel. The observed saturated attachment area after 2h is about  $33\mu\text{m}^2$ . This dynamic cell attachment area in the fractal microchannels with the similar hydraulic diameter as the single channel case also increases with time. But it

doesn't reach its saturation situation in 2h. The detailed mechanism needs clarification in the future.

#### ACKNOWLEDGMENT

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