

Chaotic vessels fabricated by fractal gelatin

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A novel gelatin patterning technique applied to making capillary vessels with fractal dendrite configuration is presented. Such a chaotic tree-like pattern has been obtained by precipitating among the gelatin matrix that is spin coated on a glass substrate at room temperature. The weight percentage of the original gelatin solution is over saturated. As the temperature decreases, the gelatin crystallises and forms a natural fractal pattern extruding the film surface. The process conditions of spin coating and the gelatine concentration are varied so that the hydraulic diameters of the fractal branches are verified from 0.1 to 23 μm . The authors therefore fabricated hollow vessels by the polydimethylsiloxane (PDMS) de-moulding of the soft lithography. The water filling test of the PDMS vessel chip is done accordingly. The issue of the toxicity is also addressed.

1. Introduction: Artificial blood vessels are important to implant surgery related to many irrecoverable damages of human organs. The material for the blood vessels should meet the requirements of good biocompatibility and long-life time. For example, poly(lactic-co-glycolic acid) nanofibre [1, 2] and polyvinyl alcohol membrane [3] have been reported as vessels materials. Other circular microchannels biocompatibly made by polymethyl methacrylate [4] and polydimethylsiloxane (PDMS) [5] were also recently reported as microvascular networks. Additional materials extracted from animal tissues or natural creatures seem more suitable than those polymer materials [1–5] to implant applications. One choice of these materials is gelatin. Gelatin poses 18 kinds of amino acid and can be shaped into different geometries or patterns in the previous studies correlated with the weak microstructure strengthening and the selective stem cell culture [6, 7]. In these prior works, the gelatin micropatterns are very regular according to the designers' mask layouts hence far from the biomimicking manner; for example, the fractal patterns discussed in this Letter. In other words, if we regard the gelatin as a material for making artificial blood vessels to mimic the chaotic vessels, the first difficulty to overcome is how to develop fractal gelatin patterns.

Fractal patterns are commonly found during the re-crystallisation process of over-saturated salty solution [8, 9]. Hence the re-crystallisation process can be regarded as an approach to form the micro- or nanostructures with fractal patterns. In [10], the authors have chosen the gelatin aqueous solution dissolved with excess amount of potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) to occasionally generate the tree-like, fractal microstructures. The classic weight percentage of the gelatin aqueous solution for generating the fractal gelatin is 10–20 wt%, with the matching amount of $\text{K}_2\text{Cr}_2\text{O}_7$ as 5–15 wt%. The prior works [10, 11] investigated to the size influence of the gelatin fractal patterns and its application to the design of micromixers. In this Letter, however, the authors would like to use gelatin fractal pattern to fabricate some chaotic capillary channels for liquid transportation and even more as a promising artificial blood vessels in the future.

2. Fabrication and experiments: The following purpose is to fabricate the fractal gelatin, PDMS chaotic vessels and to discuss the corresponding technical issues.

2.1. How to make fractal gelatin: The gelatin solution needs gentle heating up to 50–60°C with proper mixing and bubble removing. Mixing $\text{K}_2\text{Cr}_2\text{O}_7$ as a salt into the gelatin aqueous solution will

enable the gelatin crystallisation. Herein, the spin coating process is accessed to spread the gelatin film on a glass substrate. The dispensing time of the gelatin gel on the substrate should be controlled as short as possible to avoid gelatin solidification before the uniform spin coating. With the proper selection of gelatin and dichromate weight percentage, the re-crystallisation happens no sooner than the completion of spin coating. The tree-like, dendrite, fractal microstructures in gelatin are shown in Fig. 1. The width of the dendrite is no more than 40 μm ; the maximum height is about 10 μm . This salting-out of the gelatin fractal patterns is quite different from other methods of growing fractal patterns [12–17].

2.2. Process conditions of making fractal gelatin: A variety of fractal gelatin patterns corresponding to different process parameters are observed. These process parameters are the spin coating speed of 500, 1000, 1500 and 2000 rpm; the weight percentages of gelatin of 10, 15 and 20 wt% and the weight percentages of $\text{K}_2\text{Cr}_2\text{O}_7$ of 5, 10 and 15 wt%. The authors specifically evaluated the hydraulic diameters of the gelatin fractal patterns as the characteristic length. The hydraulic diameter D_h of the fractal gelatin according to different process parameters are shown in Fig. 2. Each datum value of Fig. 2 is obtained by averaging at least five measured points from one gelatin sample. The error bar of each datum is also evaluated as well. The cross-section of a fractal gelatin tree-like branch bumped and embedded in the gelatin film is regarded as a semi-ellipse herein. Therefore all the values of D_h are calculated by the elliptic cross-section formulas as (1)

$$D_h = 4A_c/P \quad (1)$$

$$P = \pi \left[3(a+b) - \sqrt{(3a+b)(a+3b)} \right] \quad (2)$$

$$A_c = \pi ab \quad (3)$$

where A_c and P denote the cross-section area and the perimeter of the flow channel under investigation. The values of a and b in (2) and (3) denote the long semi-axis and the short semi-axis, respectively, of the ellipse. They are quantitatively determined from the scanned curve of the fractal gelatin patterns by scanning electron microscopy (SEM) or the surface profiler (alpha-step 500).

From Fig. 2, the fractal gelatin dimension or the hydraulic diameter globally decreases with the increasing coating speed and the lower wt% concentration of gelatin as well as dichromate salt. In Fig. 2a, the finest hydraulic diameter of fractal gelatin branch

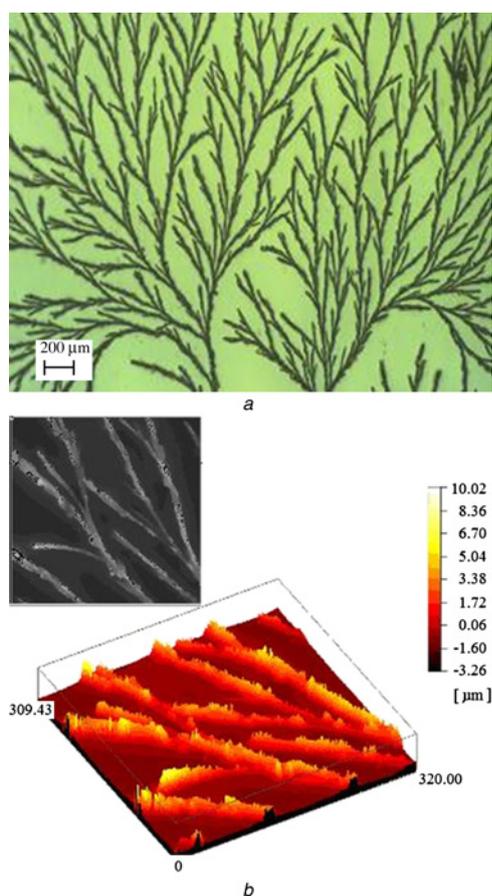


Figure 1 Classical fractal micropatterns in over-saturated $K_2Cr_2O_7$ -gelatin collagen matrix (maximum height of the pattern is $10\ \mu\text{m}$)
 a Optical microscopic photo
 b 3D morphology (NanoFocus $\mu\text{Surf RC}$, Germany)

is $0.1\ \mu\text{m}$ subject to 10 wt% dichromate in 10 wt% gelatin solution spun coating at 2000 rpm. Except in the case of 10 wt% gelatin solution in Fig. 2a, no fractal gelatin appeared with the dichromate wt% less than 5%. Additionally, the fractal gelatin dimension changing trend subject to lower wt% behaves more unpredictable in Figs. 2a and b. Their hydraulic diameters also do not match with the general blood vessels. Therefore the thicker case of 20 wt% gelatin solution in Fig. 2c is finally chosen as the candidate for the fractal pattern transfer to PDMS.

2.3. Fractal patterns transfer to PDMS: Artificial blood vessels with chaotic dendrite tree shape mimicking the portal veins of livers were proposed by Yang *et al.* [18]. So far the authors could not find a proper method to fabricate circular blood vessels directly by the gelatin material. An easier approach is to regard the fractal gelatin as a mother template, and to use the PDMS de-moulding technique to transfer the bumped fractal patterns into the concave fractal hollow channels in a PDMS block. The drawback of this method is that the cross-section of the fractal channel is only semi-circular or semi-elliptic. However the hyperelastic PDMS with large deformation capability subjected to a high pressure liquid filling could somewhat compensate this shortcoming.

The PDMS process is shown in Fig. 3. For ensuring the gelatin fractal structures are stable and remained on the glass substrate after peeling off the PDMS in Fig. 3d, UV exposure to crosslink the $K_2Cr_2O_7$ -gelatin with the fractal patterns is believed to be more robust than the pure gelatin film without crosslinking. Regarding the PDMS moulding process [19] in Fig. 3c–e, the authors mixed the PDMS base gel (Sylgard 184A) with 10:1 weight ratio to the hardening agent (Sylgard 184B) and placed

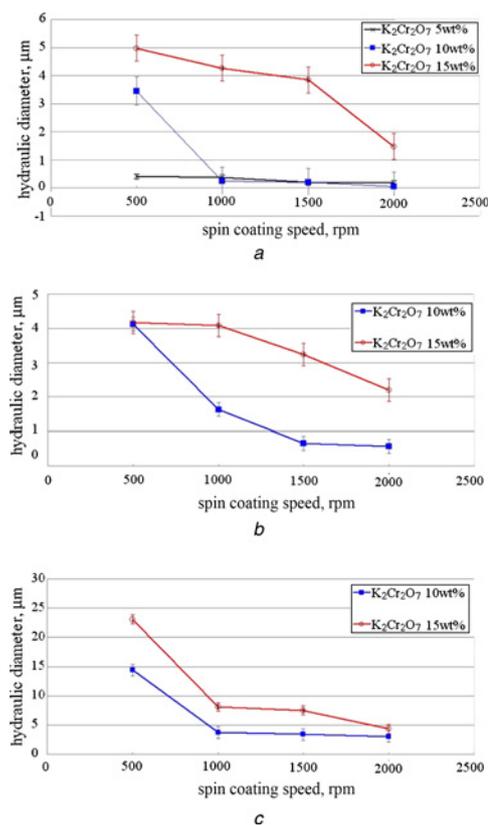


Figure 2 Hydraulic diameters of fractal gelatin against spin coating speed with respect to different dichromate weight percentages
 a In 10 wt% gelatin
 b In 15 wt% gelatin
 c In 20 wt% gelatin

the PDMS mixed gel in a vacuum chamber for 30 min to expel the trapped bubbles before the PDMS dispensing and curing. By trimming or even tailoring the PDMS slab with proper chaotic vessel patterns as well as the inlet/outlet ports on it, a hydrophilic surface treatment by oxygen plasma for 10 s (RF power of 30 W; oxygen flow of 10 sccm) by SAMCO-RIE-1C is performed. Therefore the final bonding of the PDMS slab with another glass slide as the flow channel chip is completed.

The fabricated PDMS flow chip with chaotic vessels is shown in Fig. 4. Herein the authors only passively selected proper gelatin patterns for a chaotic channel network after the growth of the fractal gelatin. The process parameters for the fractal gelatin are 20 wt% gelatin aqueous solutions with 15 wt% $K_2Cr_2O_7$ subjected to 500 rpm spin coating speed. According to the process data in Fig. 2c, the hydraulic diameter of this fractal channel is estimated to be $23\ \mu\text{m}$.

2.4. Water filling test: The fabricated PDMS flow chip is performed with water filling to confirm the mechanical strength. The volumetric flow rate of the syringe pump is set as 5–40 ml/min. The authors observed the width change of the fractal channels under the optical microscope and summarised them in Table 1. The similar viewgraphs are as Fig. 5. As the flow rate is up to 30 ml/min, the width change of 7% is observed. The corresponding pressure drop across the fractal channel is calculated as 6.24 MPa by the Hagen–Poiseuille (4) [20]

$$Q = uA_c = \frac{\Delta P \pi D_h^4}{128 \mu l} \quad (4)$$

where Q , u , μ denote the volumetric flow rate, flow speed and

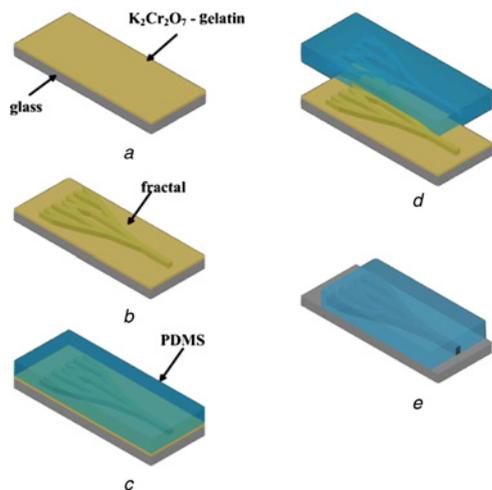


Figure 3 Process flow of PDMS fractal channels animating blood vessels
a Spin coating the gelatin film with over-saturated $K_2Cr_2O_7$
b Fractal gelatin precipitation and UV exposure
c Dispensing PDMS
d PDMS de-moulding
e Glass substrate bonded with the PDMS slide with fractal channel pattern

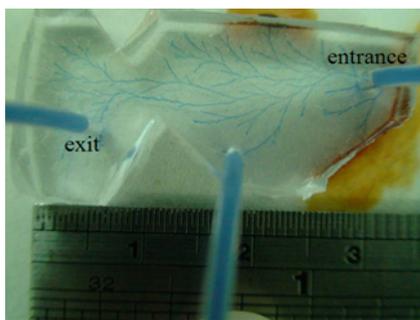


Figure 4 Filling test of the PDMS chip with chaotic vessels

Table 1 Testing results of the filling experiment on the PDMS chip

Volumetric flow rate Q , ml/min	Flow speed u , m/s	Pressure difference ΔP , MPa	Width change of fractal channels ($L = 0.035$ m; $\mu = 1.003$ mPa)
5	10.6	1.19	none (width = 100 μ m)
10	21.1	2.38	none (width = 100 μ m)
15	31.7	3.57	none (width = 100 μ m)
20	42.3	4.76	none (width = 100 μ m)
25	52.9	5.95	none (width = 100 μ m)
30	59.3	6.24	7% larger (width = 107 μ m)
35	64.4	6.30	15% larger (width = 115 μ m)
40	70.5	6.61	20% larger (width = 120 μ m)

viscosity of the fluid. L is the channel length of the flow channel. ΔP is the pressure drop across the channel length L ($=3.5$ mm observed from Fig. 4).

This pressure drop $\Delta P = 6.24$ MPa is 4.46 times of the Young's modulus of PDMS, 1.4 MPa. Therefore the apparent channel deformation is reasonable. The flow speed of 59.3 m/s is much faster than the test speed of 2 mm/s for red blood cells [21], and even

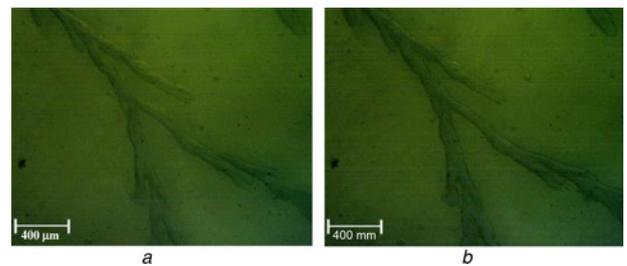


Figure 5 Water filling experiment to the fractal channel in PDMS
a Channel without water filling
b Channel filled with 35 ml/min water and the width changed with 15%

tremendously faster than the flow speed of 10 μ m/s for tumour cells in capillaries [18]. Therefore the PDMS fractal blood vessels are robust enough to be used in the general biomedical flow filling.

2.5. Toxic issue of fractal gelatin: Considering $K_2Cr_2O_7$ -gelatin is toxic and not proper for biomedical applications, the authors actually coated the gelatin surfaces with parylene between the steps *b* and *c* in Fig. 3 as the isolation and assigned them as the moulds for PDMS pattern transfer. Moreover, the authors chose other non-toxic salt of sodium chlorine (NaCl) for recrystallising the fractal patterns inside gelatin [10]. The weight percentage is 18.2 wt% for gelatin and 9 wt% for NaCl. After the measurement of surface topology, the maximum height of the pattern is not only around 2.5 μ m herein, but also the geometry is changing from the tree-like dendrite to the compound-leaf or backbone-like shape as in Fig. 6. Even though the main backbone line width of this compound-leaf fractal is about 20 μ m, similar to the tree-like dendrite case of $K_2Cr_2O_7$ -gelatin. However, there are at least three reasons so far against NaCl-gelatin as the fractal mould of chaotic blood vessels. First, the backbone fractal pattern is not like the pattern of capillary blood vessels. Secondly, the finer branches of the backbone pattern is only several μ m wide, and it

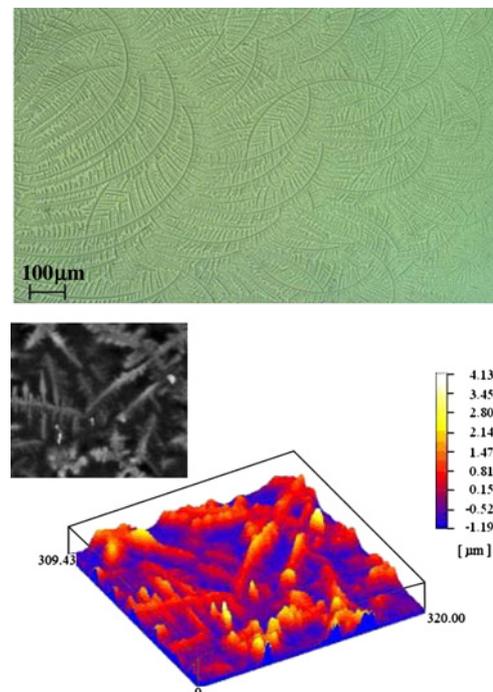


Figure 6 Fractal patterns in 18.2 wt% gelatin dissolved with 9 wt% of non-toxic NaCl (maximum height of the pattern is 2.5 μ m)
a Optical microscopic photo
b 3D morphology (NanoFocus μ Surf RC, Germany)

is too crowded to select a proper site as the inlet/outlet ports for the blood vessel network like Fig. 4. Thirdly, the NaCl-gelatin lacks the photo-sensitive crosslink mechanism like $K_2Cr_2O_7$ gelatin, and it may not mechanically strong enough to withstand the PDMS moulding process for many times. Therefore looking for other proper salts to have the non-toxic fractal pattern with the appearance like Fig. 4 will be deserving follow-up work in the future.

3. Conclusions: PDMS chaotic vessels moulded from fractal gelatin patterns have no damage issues related to MPa pressure loading so far. The characteristic hydraulic diameter of the chaotic microchannel ranges from 0.1 to 23 μm in this work. Other fractal patterns in NaCl-gelatin collagen matrix are also successful tried. The potential applications of this fractal channel network include artificial blood vessels, cell culture attachment investigation and chaotic micromixers.

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