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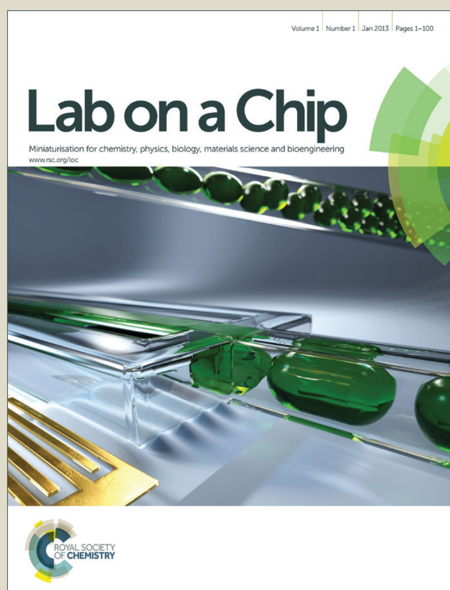

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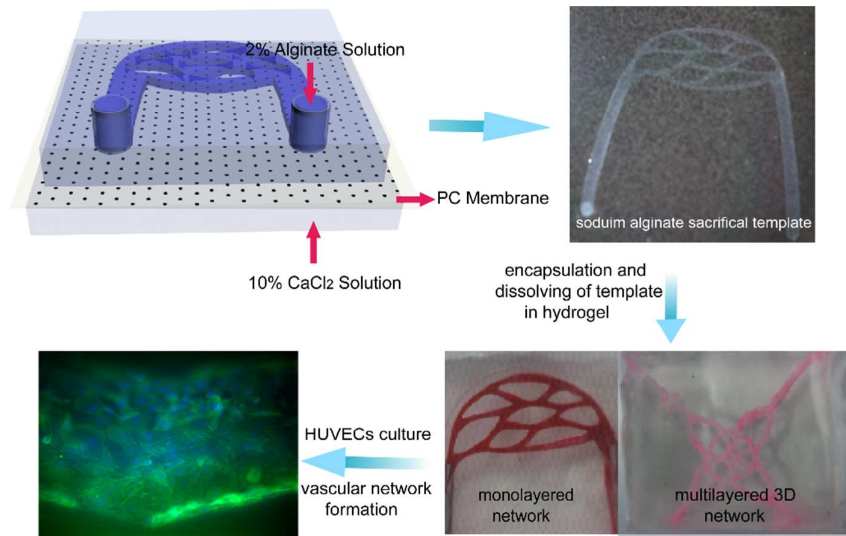

We engineer interconnected 3D microfluidic vascular networks in hydrogels using molded sodium alginate lattice as sacrificial template. Interconnected channels with well controlled size and morphology are obtained by rapid dissolving the template, which enables endothelial cells culture to form simulated vascular networks.

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Engineering interconnected 3D vascular networks in hydrogels using molded sodium alginate lattice as the sacrificial templates

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Engineering 3D perfusable vascular networks in vitro and reproducing the physiological environment of blood vessels is very challenging for tissue engineering and investigation of blood vessel function. Here, we engineer interconnected 3D microfluidic vascular networks in hydrogels using molded sodium alginate lattice as sacrificial templates. The sacrificial templates are rapidly replicated in polydimethylsiloxane (PDMS) microfluidic chips via Ca^{2+}-crosslinking and then fully encapsulated into hydrogels. Interconnected channels with well controlled size and morphology are obtained by dissolving the monolayer or multilayer templates with EDTA solution. The human umbilical vein endothelial cells (HUVECs) are cultured on the channels linings and proliferated to form vascular lumens. The strong cell adhesion capability and adaptive response to shear stress demonstrate the excellent cytocompatibility of both the template and template-sacrificing process. Furthermore, the barrier function of endothelial layer is characterized and the results show that a confluent endothelial monolayer is fully developed. Taken together, we develop a facile and rapid approach to engineer vascular model that could be potentially used in physiological studies of vascular functions and vascular tissue engineering.

Introduction

Multiscale vascular networks of the circulatory system play critical roles in complex mass transport including delivery of oxygen and nutrient to, and removal of metabolic by products from tissues. Dysregulation of such precise system will result in various kinds of diseases.[1,2] Although great successes have been achieved during the past several decades, the limitations of existing cell culture and animal studies have provided an impetus for the development of in vitro models that better mimic the complex structures and functions of living organs with vascular networks.[2,3]

During the past decade, microfluidic devices have been emerged as versatile tools to reconstitute the structural tissue arrangements and functional complexity of living organs with blood vessels.[4,5] A considerable number of models based on PDMS microfluidic channel have been successfully developed for blood vessel mimicking such as ECs culture on PDMS membranes for vascular function simulation[6-9] and construction of ECs monolayer for study of angiogenesis and tumor metastasis.[10-12] In order to generate tissue constructs similar to the structure and functions in vivo that sustained by 3D vascular architectures, various kinds of biocompatible hydrogels have been recently used as the bulk materials for microfluidic devices.[13-16] Therefore, generation of well defined 3D vascular architectures inside hydrogels is of great importance for vascular simulation and tissue engineering.[17]

So far, several techniques have been applied to form microfluidic structures in hydrogels. Single non-branching fluidic channels were formed in hydrogels by pouring the hydrogel precursor over a metal (e.g. gold and stainless steel) rod[18, 19] or glass capillary[20, 21] followed by pulling out the templates, but this method could only generate single straight lumen with uniform size and morphology. Although several techniques such as smart hydrogel technology using light to control the degradation of hydrogel components,[22] hydrogel bonding by heating[23] and direct writing[24, 25] have been developed for generation of endothelialized microfluidic vessels to simulate complex perfusable vascular networks, these approaches are more or less limited by complicated processes or expensive instruments.

In contrast to these methods, 3D sacrificial moulding provides a very promising alternative to construct 3D vascular architectures in hydrogels.[26] Studies show that a network of

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microfluidic channels can be fabricated by creating a 3D template and then sacrificing the template to reveal a microfluidic architecture in the bulk material.[27-30] However, 3D sacrificial template for perfusable channels usually involves cytotoxic organic solvents, extreme temperature or pressure for removing the sacrificial templates, and could not be accomplished widely with aqueous-based extracellular matrices (ECMs).

Here, we describe a method using crosslinked sodium alginate lattice as a biocompatible sacrificial template to rapidly cast interconnected 3D vascular networks in hydrogels. The templates was quickly replicated in PDMS microfluidic channels via Ca^{2+} ionic crosslinking, and then fully encapsulated in hydrogels. Dissolving the template with EDTA solution generates interconnected 3D microfluidics channels for ECs culture and vascular simulation. Using this method, the size and shape of the templates could be well controlled by patterned PDMS, and multilayered vascular networks with intervessel junctions could also be easily formed. The sacrificial template dissolved rapidly and biocompatibly, and the HUVECs are well adhered and proliferated on the channel linings to form a confluent endothelial layer with excellent barrier function for generation of vascular networks.

Reagents and Methods

Materials and reagents

Polydimethylsiloxane (PDMS) was purchased from Mementive (USA). Polycarbonate membrane with pore size of 0.2 μm was purchased from Whatman (UK). Sodium alginate, Anhydrous calcium chloride ($CaCl_2$), Ethylene diamine tetraacetic acid (EDTA) were supplied by Shanghai Chemical Reagent Company (China). The gelatin and agarose (with low gelling temperature) were purchased from Sigma (USA). The collagen Type I was purchased from BD (USA). Transglutaminase was purchased from Yiming Biological Products Co., Ltd (China). The cell culture medium RPMI 1640, L-glutamine and HEPES for HUVECs culture were purchased from GIBCO (USA). FITC-labeled dextran (M_w = 20 kDa), Rhodamine-labeled dextran (M_w = 70 kDa) were purchased from Sigma (USA). 3',6'-Di(O-acetyl)-4',5'-bis[N,N-bis(carboxymethyl)-aminomethyl] fluorescein, tetraacetoxymethylester (Calcein-AM), Alexa Fluor 488 phalloidin, 3,8-diamino-5-[3-(diethylmethylammonio)propyl]-6-phenylphenanthridinium diiodide (PI) and 4',6-diamidino-2-phenylindole (DAPI) for cell staining were obtained from Invitrogen (USA). Rhodamine-labelled microspheres (5-7 μm) were purchased from ACME microspheres (USA). All other chemicals unless specified were reagent grade and used without further purification. All solutions were prepared and used under sterile conditions.

Formation of molded sodium alginate sacrificial templates

Sodium alginate sacrificial template was formed in a patterned microfluidic channel. The multilayer microfluidic system comprised a structure-patterned PDMS layer and another PDMS layer sandwiching a porous polycarbonate membrane with pore size of 0.2 μm. PDMS molds were fabricated by conventional photolithography and soft lithography, sterilized with 75% ethanol, and lighted in UV/ozone over night before use. 2.0 % (w/w) sodium alginate solution was injected into the patterned PDMS layer and cross-linked with 10% (w/w) $CaCl_2$ solution that diffuse across the porous polycarbonate membrane for 30 s, therefore producing patterned sodium alginate template. Then the template was immersed into ethanol for 30 s to increase the stiffness and then separated from PDMS molds. In the following steps, ethanol in template would volatilize away and could not influence the compatibility of hydrogels.

The multilayered sodium alginate template was fabricated by welding two or more monolayered sodium alginate templates together to form an integrated 3D template. Briefly, as prepared monolayered sodium alginate templates separated from the PDMS molds were aligned and stacked up where many overlapped connection were formed between different layers. To integrate each layer, the overlapped connection were slightly dissolved with 40 mmol/L EDTA solution for less than 30 s, enabling the connection welding and forming junctions between multilayers. Subsequently, the integrated template was immersed into 10% (w/w) $CaCl_2$ solution to stop the dissolution of sodium alginate and to re-crosslink the integrated 3D template. Finally, the template was dipped into ethanol for 30 s to increase the stiffness.

Formation of microfluidic hydrogel with interconnected microfluidic channels

To form gelatin hydrogel with interconnected microfluidic channels, the sodium alginate sacrificial template was firstly transferred on a layer of pre-gelled matrix (the matrix was composed by 12.5% (w/w) gelatin and 10% (w/w) chemical crosslinking agent transglutaminase[31] with a ratio of 8:1), and two glass capillaries (0.5 mm in diameter) were horizontally placed onto the two ends of the template. Then the second liquid gel precursor was coated until the template and glass capillaries were completely immersed. After gelation of the outer matrix for 2 h at 37 °C in a CO_2 incubator (Heracell 150i, Thermo Scientific, USA), the two glass capillaries were pulled out from the gelation matrix to generate an inlet and outlet. The patterned microfluidic networks were obtained by perfusing 200 mmol/L EDTA solution from the inlet to dissolve the template. Then, the remaining EDTA solution was subsequently washed away by PBS solution and cell culture media. The collagen I and agarose hydrogel with interconnected microfluidic channels were gained with the similar procedures, but collagen hydrogel was prepared by mixing type I rat tail liquid collagen, NaOH solution, and RPIM 1640 medium at 4 °C and crosslinked at 37 °C for 30 min, while agarose hydrogel solidified at room temperature.

Formation of microfluidic hydrogel with pseudo-3D or 3D interconnected microfluidic networks

The pseudo-3D multilayer branched microfluidic networks were constructed by encapsulating and dissolving isolated templates inside hydrogels. Firstly, the sodium alginate template was transferred on a pre-gelled hydrogel and then the second liquid gel precursor was coated until the template was completely immersed, after partial gelation of this hydrogel for 10 min, another sacrificial template was transferred on the hydrogel and the steps was repeated until all the templates were encapsulated on different layers and gelation for 2 h. The templates of different layers were dissolved by 200 mmol/L EDTA solution. The different layers of microfluidic networks could be either completely isolated with independent inlets and outlets or be connected by a common outlet.

Multilayered 3D microfluidic networks in hydrogel were obtained by dissolving the multilayered 3D sodium alginate sacrificial template with the similar steps as fabrication of monolayered microfluidic channels. Then, the inlet of bottom

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layer and the outlet of top layer were connected with stainless steel needles and elastic tubing. The fluid injected from the bottom channels would flow into the top network via the junction of the microfluidic channels, thus an enclosed multilayered 3D connected flow network was formed.

Scanning electron microscopy

The templates were frozen at -20°C and then lyophilized. The surface of the template was sputtered with gold, and observation of the morphology was carried out on a field emission-scanning electron microscope (FE-SEM, Sirion Tmp, Fei Co. USA).

Human endothelial cells culture

The human umbilical vein endothelial cell lines (HUVECs) (XiangYa Central Experiment Laboratory, China) were routinely cultured using RPMI 1640 culture medium with 12 % fetal bovine serum(FBS), 0.292 mg/mL L-Glutamine, 4.766 mg/mL HEPES, 0.85 mg/mL $NaHCO_3$, penicillin and streptomycin (100 U) in the culture flask. The cells were cultured for 4 days and then suspended in fresh medium with a density of 1×10^6 cells/mL. The elastic tubing and stainless steel needles were sterilized prior to use, and the networks in hydrogel were washed with fresh RPMI 1640 medium and the HUVECs were slowly perfused into the microfluidic network. After adherence, the HUVECs were cultured on the inner surface of the channels in a CO_2 incubator.

Fluorescent staining and cell imaging

The viability of cultured cells was checked by Calcein-AM and PI. 10 μL Calcein-AM (1mg/mL) and 15 μL PI (1mg/mL) solution were diluted with 5 mL PBS solution and used for cell staining. Cells were stained as follows: Firstly, the cells in microfluidic network in hydrogel were washed three times with pH 7.4 PBS solution and the medium was replaced with PBS solution; Then, Calcein-AM and PI solution were slowly injected into each channel of network and the hydrogel chip were maintained in a humidified incubator (37 °C, 5% CO_2) for 30 min; Finally, the Calcein-AM and PI solution was replaced with PBS solution and washed more than three times. The fluorescence imaging of stained endothelial cells was observed under an AxioObserver Z1 fluorescent microscope (AxioObserver Z1 fluorescent microscope with camera and incubation system, ZEISS, Germany, Excitation and emission wavelength were 488 nm and 544 nm respectively).

For immunofluorescence staining of F-actin and nuclei in HUVECs, cells in the patterned vascular network were washed twice with pH 7.4 PBS solution and fixed in situ with 4%(w/w) paraformaldehyde for 30 min at 4°C. Then, the hydrogel was placed in a petri dish and extracted with a solution of 0.1% Triton X-100 in PBS solution for 30 min. 1% bovine serum albumin (BSA) was added to the staining solution to reduce nonspecific background staining. 25 μL methanolic stock solution of Alexa Fluor 488-conjugated phalloidin (1:40; Invitrogen) and 40 μL DAPI (250 μg/mL, Invitrogen) were mixed into 1.0 mL PBS for each hydrogel chip and the cells were stained for 30 min at room temperature. Nuclei were labelled with DAPI, F-actin was labelled with Alexa Fluor 488-conjugated phalloidin. The fluorescent images are obtained by an AxioObserver Z1 fluorescent microscope at 25 °C.

Flow generation and pressure tolerance characterization

The flow of Rhodamine-labelled microspheres (5-7 μm) and cell culture media through microfluidic networks in hydrogel were generated by a TS-2A pump (constant pump Co., Ltd. Baoding Lange). Internal pressure of the network was measured by filling the channels with water, keeping one end of the channels closed. Internal pressure of the channels was increased with continuous perfusion of water into the network, and the pressure was recorded with a digital manometer (Nanjing Helm Sci-tech Co. Ltd, China).

Shear stress evaluation

The physical properties such as the cell adhesion were studied under the flow conditions. The shear stress τ_w was calculated by: $\tau_w = 6\eta Q/wh^2$, where Q is the flow rate, η is the fluid viscosity, h and w are the height and width of the channels respectively. The HUVECs were perfused into the microfluidic networks and adhered on the lining of channels, then cultured for 24 h under static or flow conditions with the shear stress of 1 dye/cm^2, respectively. The distribution of the orientation angle in the absence and presence of shear stress are quantitatively analysed. Different shear stresses were introduced into the vascular networks to test the cell adhesion ability after the HUVECs were cultured under the static condition for 24 h.

Characterization of endothelial monolayer barrier function

To quantify the barrier function of the fully-developed endothelial layer, the diffusion of soluble factors with different molecular weights (FITC-labeled dextran, M*w* = 20 kDa and rhodamine-labelled dextran, M*w* = 70 kDa) in gelatin were investigated. The fluorescent dextran was mixed with culture media at a concentration of 20 μg/mL, and then injected into the vascular network. The fluorescent images were obtained by an automated live cell imaging system. The diffusive permeability coefficient P_D of the endothelial barrier was calculated according to previously reported method[14]:

$$P_D=\beta\cdot D\,(dC/dx)/\Delta C;$$

Where C is the dextran concentration (proportional to fluorescence intensity), β is an area correction factor (~ 1), ΔC is the step drop in concentration across the monolayer, dC/dx is the slope of the concentration profile, and D is the dextran diffusion coefficient inside the ECM. The diffusion coefficients of the 20 kDa and 70 kDa dextrans were assumed to be 6.5×10^{-11} m^2/s and 4.5×10^{-11} m^2/s based on a scaling law for soluble factor diffusion in water. The fluorescent intensity of images were analysed to obtain the concentration gradient (dC/dx) and step drop in concentration (ΔC) across the endothelium.

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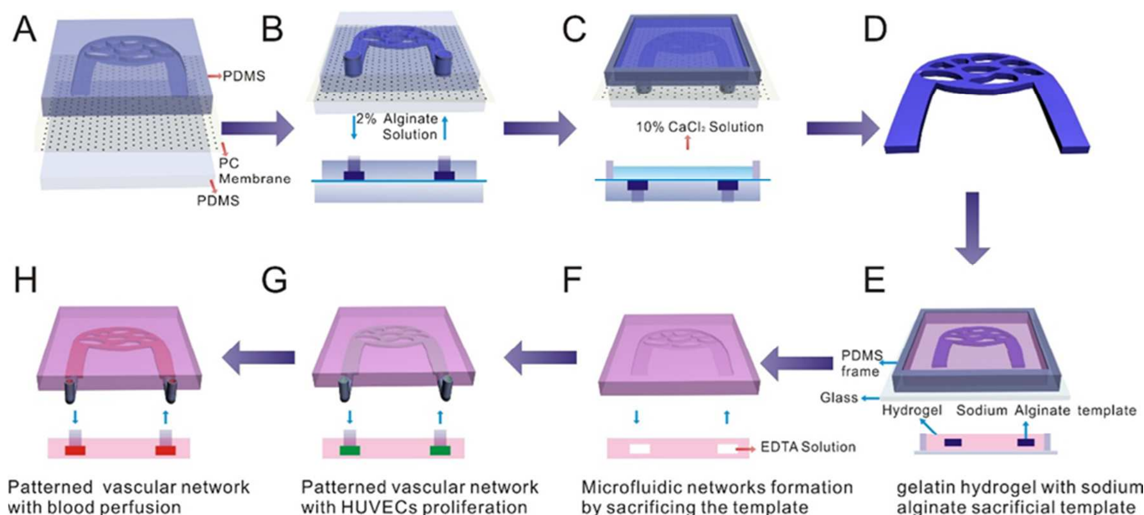

Fig. 1 Schematic diagram showing the fabrication of sacrificial template and casting of patterned vascular networks.(A-C) Multilayered microfluidic system comprised of a structure-patterned PDMS layer (sodium alginate layer) and another PDMS layer ($CaCl_2$ layer) sandwiching a porous polycarbonate membrane; (D) As-prepared sodium alginate sacrificial template; (E) Encapsulation of the template in a hydrogel precursor, and gelation of the precursor; (F) Microfluidic network formation by sacrificing the template with EDTA solution; Patterned vascular network formation with HUVECs proliferation (G) and blood perfusion (H).

Results and Discussions

Engineering vascular networks in hydrogel

As a polysaccharide found naturally in brown seaweed, sodium alginate can be ionically crosslinked with divalent cations such as calcium to form a hydrogel, which can be reversibly dissolved with EDTA solution owing to the strong chelation interaction between Ca^{2+} and EDTA.[32] As a promising biocompatible hydrogel material, sodium alginate has been widely used as 3D scaffolds for cell culture and tissue engineering.[33,34] Here, precisely molded sodium alginate sacrificial templates were firstly fabricated by Ca^{2+}-crosslinking in patterned microfluidic channels.

Fig. 1 shows the main process for fabrication of sacrificial template and casting of patterned vascular networks. The replication of sodium alginate lattice is performed by a multilayered microfluidic system comprised of a structure-patterned PDMS layer (sodium alginate layer) and another PDMS layer ($CaCl_2$ layer) sandwiching a porous polycarbonate membrane (Fig. 1A-C). Alginate solutions with different concentrations have been used in our experiment. The alginate with concentration less than 1.0 % (w/w) is too dilute to form a solid-state template, while alginate with concentration over 5.0 % can not be easily injected into PDMS channels due to their high viscosity. Therefore, to obtain a template with suitable stiffness, 2.0 % (w/w) sodium alginate solution in the PDMS channel was cross-linked with Ca^{2+} that diffuse across the porous polycarbonate membrane, therefore producing patterned sodium alginate template (Fig. 1D, Fig. 2A). The crosslinking process is very fast and the template could be obtained in less than 30 s. Most importantly, the size and shape of the template can be well and precisely replicated by the patterned structure in PDMS stamp, which will greatly facilitate the casting of patterned vascular networks with controlled architectures. To increase the stiffness of template for subsequent manipulations, the sodium alginate template was immersed into ethanol for 30 s (ethanol will volatilize away in the following steps).

To obtain patterned microfluidic networks inside hydrogels, the sodium alginate sacrificial template was transferred into pre-gelled matrix (Fig. 1E, gelatin, agarose and collagen have been used in this work). The effects of gelatin/agarose/collagen concentrations to prepare the platforms were studied. To obtain hydrogel matrix with both sufficient mechanical stiffness and appropriate crosslinking rate, gelatin (12.5%, w/w), agarose (1.0%, w/w) and collagen (2.5 mg/mL) were finally used as the hydrogel matrix (the agarose and collagen-based hydrogel for engineering vascular networks are shown in Fig.S1 B, C). After gelation of the matrix, the patterned microfluidic network that faithfully replicated the features of templates could be easily obtained by sacrificing the template with EDTA solution (Fig. 1F, the remaining EDTA solution was subsequently washed away by PBS solution and cell culture media). The release of the sodium alginate is rapid (in less than 2 min) and compatible for cell culture.

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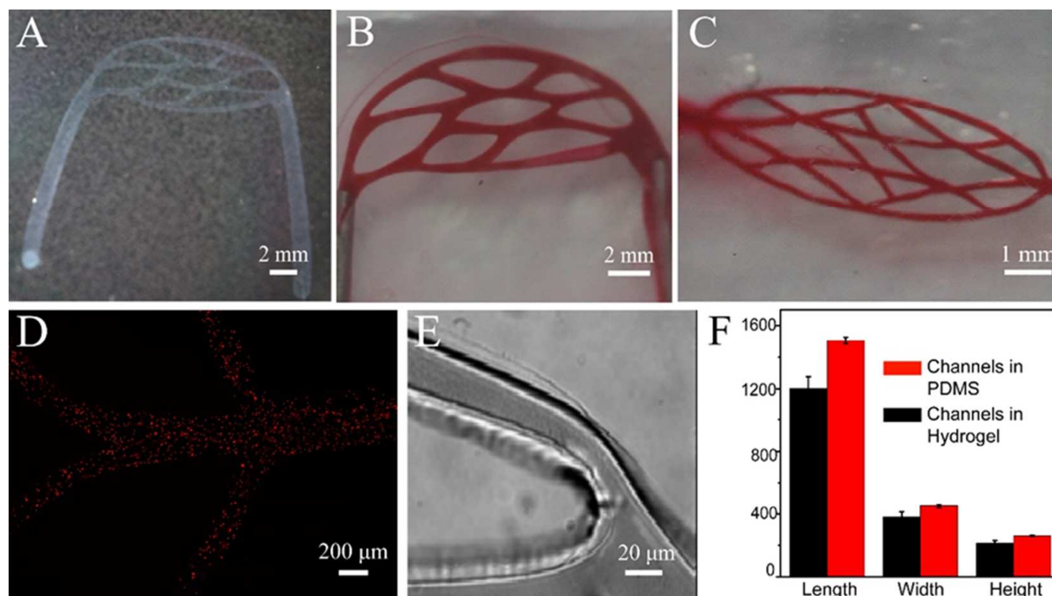

Fig. 2 Characterization of microfluidic networks in gelatin. (A) Pictures of sodium alginate template; (B, C) Pictures of microfluidic networks with different shapes and sizes in gelatin filling with red dye.(D) Fluorescence image of channels in gelatin perfused with microspheres. (E) Image of 20 µm wide channels. (F) Comparison of microfluidic network dimensions in PDMS and in gelatin hydrogel.

Characterization of the microfluidic network

The rapid flow of red dyes (Fig. 2B, C, Movie S1) and 5 µm diameter fluorescent microspheres (Fig. 2D, Movie S2) demonstrated that well defined microfluidic networks inside hydrogel have been successfully casted. This method could gain microchannels as narrow as 20 µm (Fig. 2E). Comparison of microfluidic network dimensions in PDMS and in gelatin hydrogel indicated that the mean percentage of shrinkage of the alginate is approximately 18% (Fig. 2F, this change was possibly caused by the contraction of template in ethanol), and we can adjust the size of the initial PDMS molds to get microfluidic networks inside hydrogel matrix in a controlled way. Currently, we could engineer microfluidic channels with wide range of dimensions (usually 20 µm to 500 µm in height and 20 µm to 500 µm in width) inside various hydrogel matrices, which cover most parts of vasculatures. The results indicate that hydrogel with complex microfluidic networks generated by this method may meet the needs for in-vitro blood vessel simulation and vascular tissue engineering.

To test the mechanical characteristics of the microfluidic networks inside gelatin, internal pressure of the microfluidic networks was increased up to 200 mmHg by filling the channels with water, keeping one end of the channels closed. Under these conditions, we have never observed fracturing of the channels (indicated by massive leaking of water).The value indicates that these microfluidic channels in hydrogel were robust enough to mimic blood vessels in vivo with physiological pressures and study structure and function of endothelial cell under the normal hemodynamic conditions.

Characterization of microfluidic hydrogel with pseudo-3D or 3D interconnected microfluidic networks

To mimic the microenvironment with 3D vascular networks similar to the cells growth required in vivo, we co-encapsulated three sodium alginate template at the different vertical positions and obtained pseudo 3D microfluidic vascular networks in a hydrogel chip (complexity of the network could be increased by gelled more layers). The multiple layers of microfluidic networks are independent but with a common outlet, capable of studying different effects of various factors on the cells under adjusted conditions.

Furthermore, we created interconnected 3D vascular networks in hydrogels by using multilayered lattice of sodium alginate as the template (Fig. 3B-G). The multilayered lattice was obtained by slight dissolution of the multiple templates with EDTA and re-crosslinking with Ca^{2+}, and the intervessel junction could be therefore well fused together to form an integrity of 3D template with numbers of interconnected junctions (Fig. 3B-D). An important advantage of this approach is that the perfusable scaffold is easily formed as a continuous phase simply by washing away the interconnected 3D sacrificial template in the matrix (Fig. 3E-G, Movie S3). Relying on simple chemical principles and PDMS molds rather than complicated engineering processes, this work provides an easy, rapid, inexpensive, and highly controllable technique to obtain the in vitro 3D vascular networks similar to the native blood vessels.

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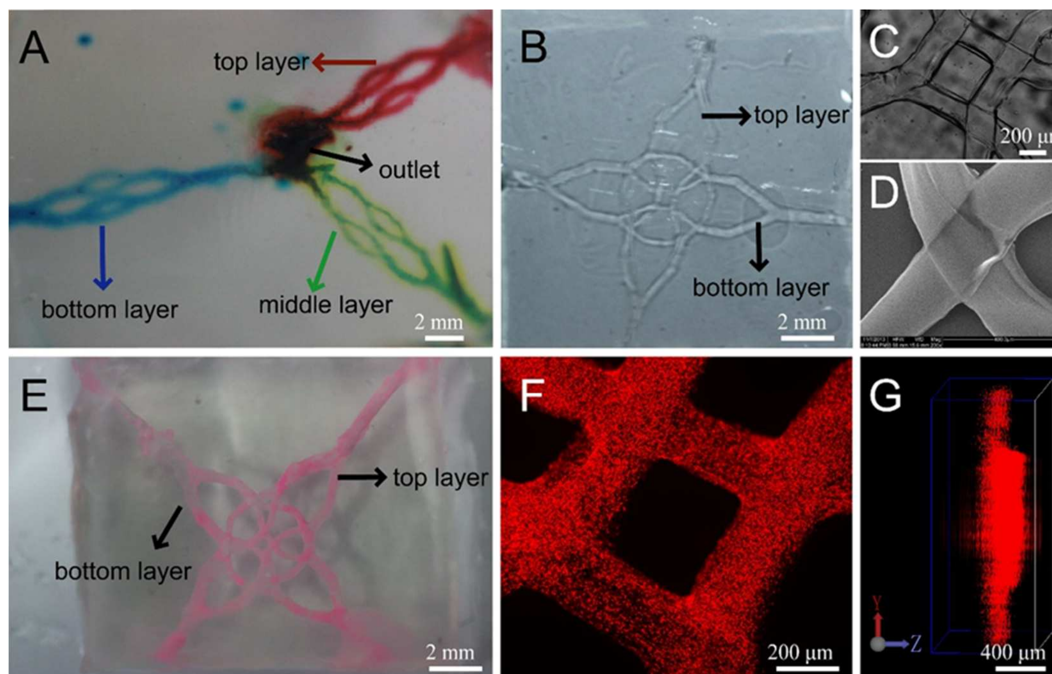

Fig.3 Creation of multilayered 3D microfluidic networks in gelatin. (A) Image of a hydrogel chip with three layer microfluidic networks on different layers; (B) Image of two interconnected layers template; (C, D) Microscope and SEM images showing the intervessel junctions between two layers of templates; (E-G) Perfusion of microspheres inside 3D interconnected microfluidic networks in gelatin.

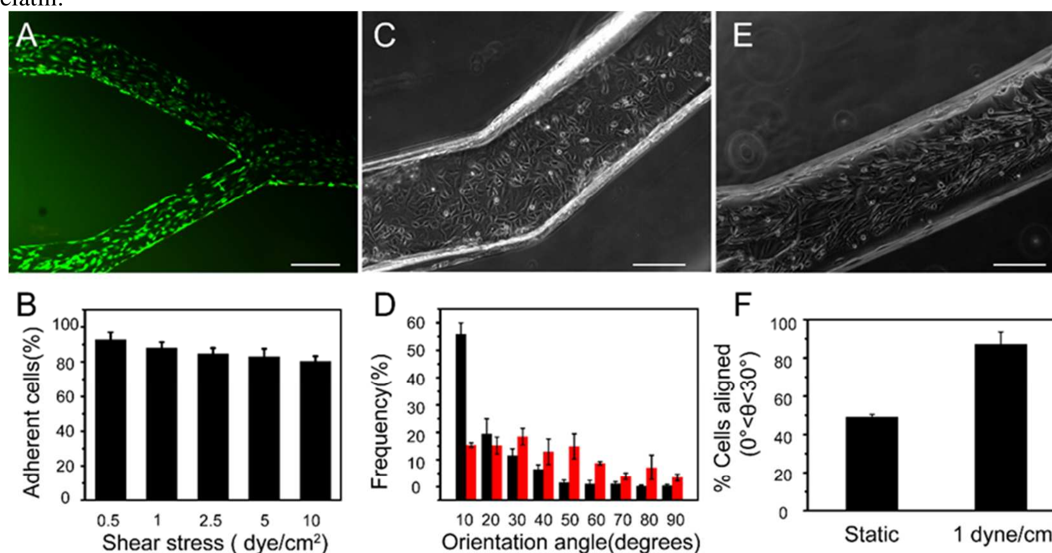

Fig.4 .The adherence strength and adaptive response of HUVECs to shear stress. (A)Microscopic picture showing the viability of the proliferated HUVECs under the shear stress of 1 dye/cm^2 after 24 h, Calcein-AM (green), PI (red);(B) Adherence of HUVECs under different shear stress after 1.0 h; (C, E) Microscopic pictures showing HUVECs cultured in the channels linings for 24 h under static condition(C) and under flow condition with the shear stress of 1 dye/cm^2 (E); (D) Quantitative analysis showing the distribution of the orientation angle in the absence and presence of shear stress. The orientation angle (θ) is defined as the angle between the major axis of the best-fit ellipse around a cell and the direction of flow; (F) adaptive response of HUVECs to shear stress, cells with orientation angles between 0° and 30° were considered aligned. Scale bars: 200 μm.

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Characterization of the adherence strength and adaptive response of HUVECs to shear stress

When a dense suspension of HUVECs was seeded through microfluidic networks in hydrogel, HUVECs could adhere to the inner wall of the hydrogel microchannels and well spread, indicating the excellent cytocompatibility of both the template and template-sacrificing process. The viability of the proliferated HUVECs under the shear stress condition of 1 dye/cm^2 after 24h was checked by injection of calcein-AM and PI from the inlet of the network to label cells, and a viability of over 99% was obtained (Fig. 4A). The adhesion ability of HUVECs onto the microfluidic network lining and their hemodynamical characteristics such as adherence strength and adaptive response to shear stress were further investigated. The HUVECs exhibit strong adherent ability on the gelatin surface under different shear stress. Under the shear stress of 10 dyne /cm^2, only 20% of the cells detached from the lining surface after 1.0 h(Fig. 4B), indicating the capability of this model in vascular simulation of veins and arteries with shear stresses in the range of 3–13 dyne/cm^2.

The shear stress induced alignment of HUVECs under flow condition was also studied. Compared with the cells cultured under static condition, the HUVECs aligned parallel to the direction of the flow and elongated to different aspect ratios under the continue effects of shear stress (Fig. 4C-F). Quantitative analysis showed a uniform distribution of the orientation angle after culturing under static conditions for 24h while the orientation angle of 86% of the cells was less than 30° after culturing for 24h under a flow condition with the shear stress of 1 dyne/cm^2.

Characterization of endothelial monolayer barrier function

After several days (usually 3-4 days depending on the initial density of ECs) of culture, seeded cells well proliferated and covered all the gelatin and collagen lining and grew along the wall to form fully-developed endothelial layer, while ECs cells on agarose gels appeared to grow as multicellular spheroids, (Fig.S1F) which was similar to previously reported work[35]. The microscopic images stained by Alexa Fluor 488 phalloidin and DAPI demonstrate HUVECs confluent monolayer coating the inside of the microfluidic channels was fully developed, generating patterned vascular networks inside biocompatible hydrogel matrix (Fig.5A, Fig. S1D, E, and Movie S4).

In order to further demonstrate that a fully-developed endothelial layer was formed, endothelial monolayer barrier function was characterized by injection of fluorescent conjugated dextran into vascular networks, and a diffusion based solute flux across the endothelial monolayer (Fig.5B, C) was used to measure the diffusive endothelial permeability (P_D). The intensity profiles were measured and the sharp drop of dextran concentration across the endothelial monolayer and the steady diffusive flux inside the gelatin, indicating the formation of fully-developed endothelial layer. P_D was measured, yielding values of $4.05 \pm 0.21 \times 10^{-5}$ cm/s and $0.66 \pm 0.082 \times 10^{-5}$ cm/s for 20 kDa and 70 kDa dextrans with a P_D ratio of 6.2(Fig. 5D), further indicating that the endothelial monolayer within our model forms a size-selective barrier.

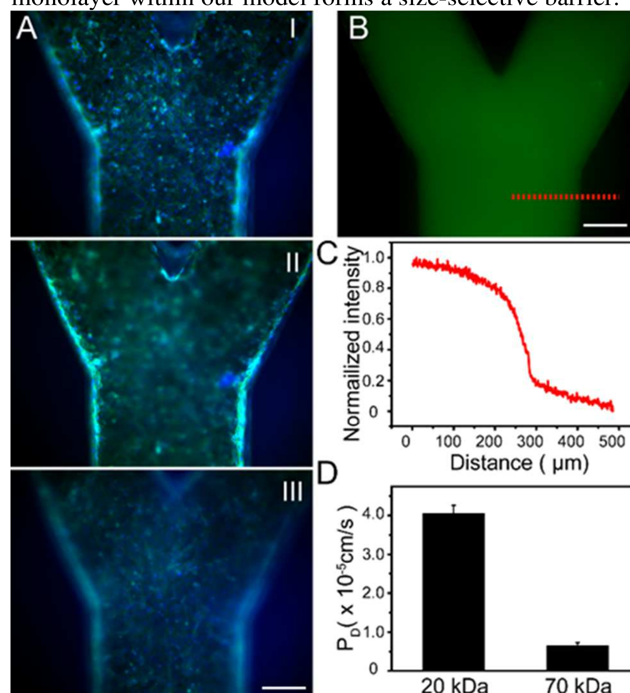

Fig.5 Characterization of barrier function of a fully-developed endothelial layer. (A) Microscopic pictures of HUVECs cultured in gelatin channels after 3 days under static conditions retracted from Z-stack model, (I) bottom layer (II) middle layer (III) top layer; Alexa Fluor 488 phalloidin (green); DAPI(blue); (B) FITC-labeled dextran (20 kDa) in the channels with endothelial layer; (C) Fluorescent intensity profile along the dashed line in B illustrating the drop of dextran concentration across the endothelial monolayer (D) Diffusive permeability (P_D) of endothelial monolayer for 20 and 70 kDa dextrans.

Conclusions

In summary, we describe a strategy for rapid casting of patterned vascular networks in ECM gels by using molded sodium alginate lattice as the biocompatible sacrificial templates. Coupled with advances of microfluidic device, this approach provides a facile, rapid, inexpensive and highly controlled technique to obtain single layer or 3D vascular networks similar to the native blood vessels. The strong adherence ability and adaptive response of HUVECs to shear stress as well as the barrier function of confluent ECs layer demonstrate the excellent cytocompatibility of both the template and the template-sacrificing process. Furthermore, the approach allows patterning arbitrary vascular networks in a wide variety of synthetic and natural extracellular matrices. This strategy described here demonstrates a promising technique for rapid formation of in-vitro vasculatures, which would enable a versatile and flexible platform for a wide array

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of specific applications such as vascular structural and functional simulation, tissue engineering and drug screening.

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