Materials Horizons

*Electronic Supplementary Information (ESI)*

# Microscopic Hollow Hydrogel Springs, Necklaces and Ladders: A Tubular Robot as a Potential Vascular Scavenger

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## 1. Supplementary Methods

### 1.1 Single- and double-helical spring-like SA hollow microfibers.

SA powder (2 g, $M_w \approx 50{,}000$, purchased from Sinopharm Chemical Reagent Co., Ltd, China) was dissolved in 70-mL deionized water under vigorous stirring by a magnetic stir bar at 80 ℃ for 4 h. The aqueous SA solution was cooled down to 25 ℃ prior to microfluidic tests, and the final concentration was 28 mg/mL.

For preparation of single-helical microfibers, a microfluidic device was designed as shown in Figure 1a. the SA solution was filled into an injector (V = 20 mL) equipped with a single needle nozzle (internal diameter: 0.7 mm). The nozzle was immersed into 2-mm depth below the aqueous reaction mixture that was prepared according to an improved method from our previous report.[33] Typically, $CuSO_4$ (5 mmol, Sinopharm Chemical Reagent Co., Ltd, China) was dissolved into 100 mL Tris–HCl buffered solution at pH 8.5 (Tris: 5.0 mmoL, HCl: 1.5 mmoL) under stirring with a magnetic stir bar at 25 ℃. With dropwise addition of $H_2O_2$ by a dropper (40 mmol, 30 wt% in water, supplied by Sinopharm Chemical Reagent Co., Ltd, China), plenty of gas bubbles were generated from the buffer solution. The SA solution was extruded into solid microfibers that entered into the buffer solution and turned into hollow structures. The SA extruding rate was controlled at 500 μL/min by a speed-tunable syringe pump (model: LSP04-1A). The diameter of microfibers was dependent on internal diameter of the nozzles (internal diameter of nozzle: 0.24, 0.30, 0.40, and 0.60 mm).

The device for formation of double-helical spring-like microfibers was designed in Figure 1b, where two needle nozzles were assembled side-by-side, and kept at 2-mm depth below the aqueous reaction mixture. The SA concentration and extruding rate were still constant at 28 mg/mL and 500 μL/min, respectively. Other operating processes were the same as these in preparation of single-helical microfibers.

**1.2 Necklace- and ladder-like SA hollow microfibers.**

The device in Figure 1b was also used for creation of necklace- and ladder-like microfibers, where the two needle nozzles were kept above the liquid level. As the distance between nozzles and liquid level was 2.0 ± 0.2 mm. The SA solution was extruded into a necklace-like solid microfiber that entered into the reaction mixture of Tris–HCl, $CuSO_4$ and $H_2O_2$ and formed a hollow necklace-like microfiber. The SA extruding rate was verified between 100 and 400 μL/min to generate hollow hydrogel

necklaces with different dimensions.

With keeping all other parameters constant, adjusting the distance between two nozzles and liquid level to 1.0 ± 0.2 mm resulted in the formation of ladder-like microfibers. The characteristics of ladder-like microfibers could be controlled by variation of the SA extruding rate from 100 to 300 μL/min.

**1.3 Hollow hydrogel microrobots.**

The hollow SA microfiber was cut to 2-mm long microtubes that were then immersed in an aqueous mixture of magnetic particles of $Fe_3O_4$ (average diameter: ~2.5 μm) for 10 s. The magnetic particles were adsorbed into the microtubes and contributed to magnetized hydrogel microtube robots in Figure 5a, C, D and F. The magnetic particles of $Fe_3O_4$ were prepared according to the reported method.[37] To obtain microrobot with asymmetrical distribution of magnetic particles in Figure 5b, one end of a 2-mm long microtube was immersed into the mixture of magnetic particles for 10 s. This operation allowed magnetic particles to be adsorbed into only one end of the microtube.

We examined controlled motility of magnetic particles-loaded hydrogel microrobots in aqueous system, where a cylindrical magnet was kept over the microrobot to generate a driving force by magnetic field interaction. The density of magnetic fields fluctuated at the range of 100 to 200 mT. A hand-held digital microscope (Model: HVS430W) was used to record the motions.

**1.4 Standard deviation (s.d.).**

A minimum of three samples were measured, and s.d. was calculated as the formula:

$S = \sqrt{\frac{1}{n-1}\sum_{i=1}^{n}(xi - \bar{x})^2}$, where $\bar{x}$ is the average value of the measured results from $x_1$ to $x_n$, and n = 3.

**1.5 Tensile tests.**

The mechanical properties of hollow necklace-like microfibers were characterized on a tensile tester (Model: HY-0580, Shanghai hengyi precise instrument limited

company) at 25 °C. The sample was clamped by the grips of movable and stationary fixtures in a screw-driven device. A load cell of 50 N was used. Newly-prepared hollow necklace-like microfibers were kept in air for spontaneous dehydration of 30 min prior to the tensile test. The gage length was fixed to 40 mm and the sample was stretched at a rate of 5 mm/min. We calibrated the load cell and extensometer before use. The tests complied with rules specified by the international standard norms. The average value and s.d. were calculated from three samples.

**1.6 Scanning electron microscopy (SEM).**

A 1-cm long SA hollow microfiber was dried in a freeze-dryer (Model: ED-1A-50, Shanghai Bilon Instrument Manufacturing Co., Ltd). It was then cut up, and the fragments were attached to a silicon wafer with adhesive carbon tape and coated with 5-nm thick gold layer. The inner and outer surfaces were observed by scanning electron microscope (S-4800, SYST TA PRO1156) with primary electron energy of 2 kV. The micrographs were collected at a pressure of $8.8 \times 10^{-7}$ Pa.

**1.7 Cytotoxicity test**

**Materials:** SA hollow microfibers, RAW264.7 cells (purchased from Cell Resource Center, Life Sciences School of Chinese Academy of Sciences, Shanghai, China). DMEM medium with/without phenol red, fetal bovine serum and pancreatic enzyme (Gibco, USA); penicillin and streptomycin (Amersco, USA); alamarBlueTM (AbD Serotec, UK); staursporine, STS (Sigma); Other reagents were ordered in China. Incubator with CO2 atmosphere, Biosafety cabinet (Thermo, USA); Ix2-ill100 inverted microscope (Olympus, USA); Synergy HT multifunctional enzyme marker (Bio-Tek, USA).

**Preparation of samples:** SA microfibers were cleaned 5 times with deionized water and then soaked in water overnight. The microfibers floating on water were collected, and dried at 50 ℃. The dried microfibers were dissolved in PBS (phosphate buffered saline) to prepare the solution at the concentration of 10 mg/mL. The aqueous solution was filtered with sterile filter membrane (pore size: 0.22 μm), and then kept in a sterile centrifuge tube for the next use.

Cell viability assay by alamarBlueTM method: The RAW264.7 cells were prepared

into suspension of the phenol red-contained DMEM with the number-average concentration of 5 × 105 cells/mL. The suspension was transferred into a 96-well plate with keeping 200 μL per well. After that, the 96-well plate was moved into a constant-temperature incubator (37 °C, 5 % CO2). For the cell viability assay, there were totally seven groups, including SA samples at various concentrations of 50, 75, 100, 150, and 200 μg/mL, the negative control group (PBS), and the positive control group (1 μM STS, Staurosporine). Each group was treated with DMEM medium without phenol red, and incubated for 72 h in the constant-temperature incubator. Then the medium was removed out and 180 μL of colorless 1640 medium and 20 μL of 0.1 mg/mL alamarBlueTM reagent were respectively added into each group. After 3 h of incubation, the absorbance at 570 and 600 nm was measured by a microplate reader, and RAW264.7 cells viability or proliferation rate was calculated according to the formula:

$$\text{Proliferation rate} = \frac{117216 \times D_{570} - 80586 \times D_{600}\,(sample)}{117216 \times D_{570} - 80586 \times D_{600}\,(control\ group)} \times 100\,\%$$

where D570 and D600 were the density of the absorbance at 570 and 600 nm, respectively.

## 2. Supplementary Figures

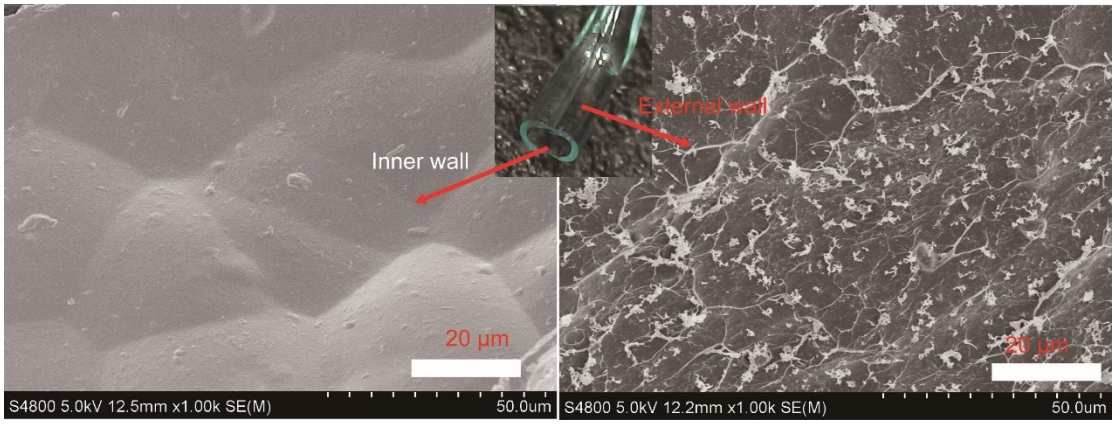


**Fig. S1** SEM images of sodium alginate hydrogel microfibers showing the surface morphologies of inner wall and external wall.

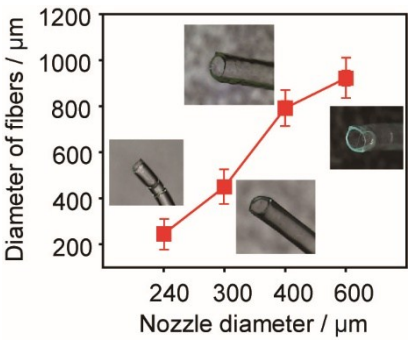


**Fig. S2** Nozzle effects on diameter of formed hollow microfibers. With keeping constant flow rate of the sodium alginate fluid, the diameter of microfibers increased with an increase of the diameter of nozzle. The s.d. from three measurements are shown as error bars.

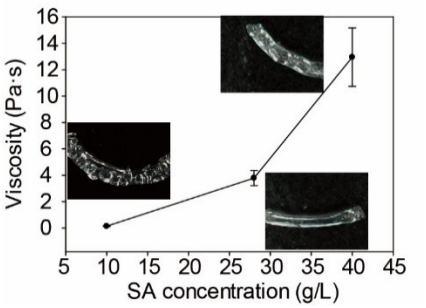


**Fig. S3** Viscosity effects on the morphologies of SA hydrogel hollow tubes. The viscosity of 0.5 Pa.s (SA concentration: 10 g/L) resulted in very soft microfibers with intermittent channel structures. Increasing viscosity to 13 Pa.s (SA concentration: 40 g/L) produced interconnected hollow microfibers, but the external surface of microfibers was not smooth. The viscosity at 4 Pa.s (SA concentration at 28 g/L) was optimal to form hollow microfibers.

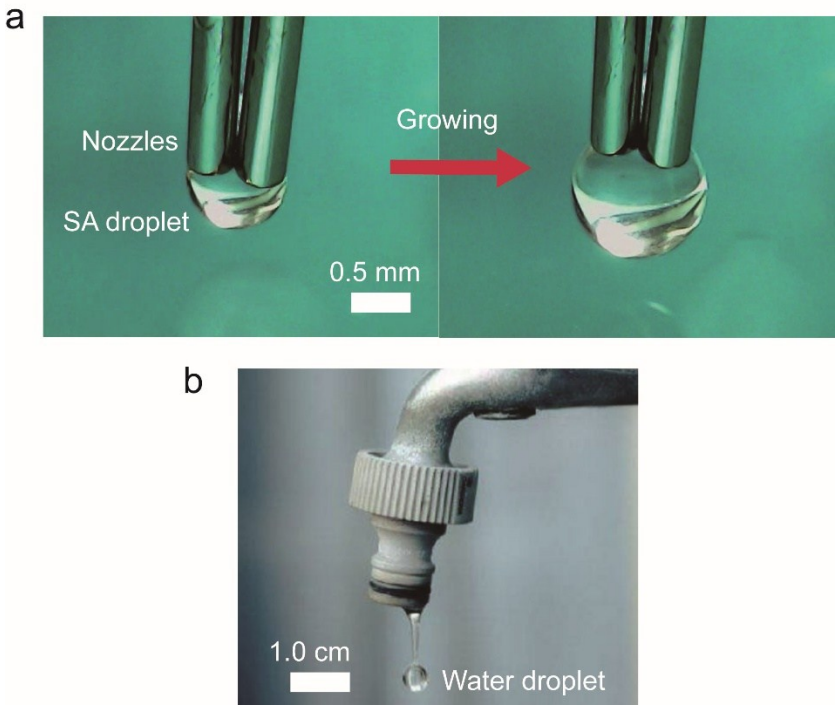


**Fig. S4** Demonstration on accumulation and growth of viscous liquid droplet. (a) Sodium alginate (SA) solution accumulated into a spherical droplet at the nozzle before entering into tris-HCl buffer solution of $CuSO_4$ and $H_2O_2$. (b) A water droplet was formed when the flow rate of water was low enough. This phenomenon inspired us to design a two-nozzles microfluidic device for preparation of necklace-like microfibers.

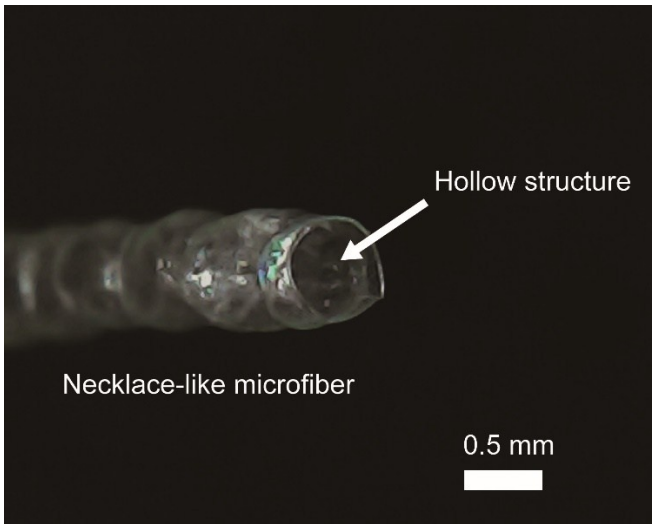


**Fig. S5** High-resolution image showing necklace-like geometry and hollow channel structure of sodium alginate hydrogel microfiber.

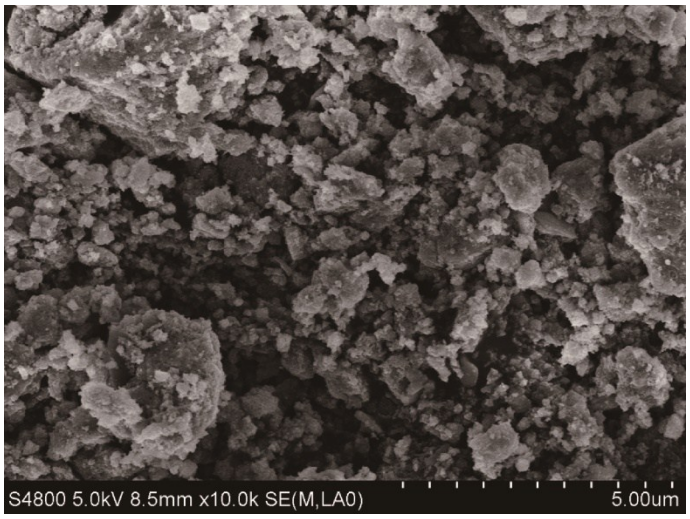


**Fig. S6** SEM image of magnetic particles of $Fe_3O_4$.

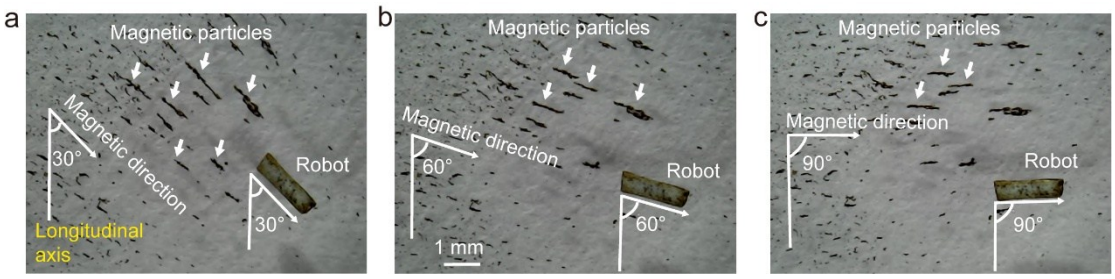


**Fig. S7** Controllable magnetic field for the motility of small-scale tubular robots. A simple device was assembled in which magnetic $Fe_3O_4$ particles (average diameter: 200 μm) were utilized as reference objects which were capable of indicating the direction of magnetic field. A magnet was fixed over a control console that could be adjusted to shift along x, y and z axis respectively to alter the direction of magnetic field.

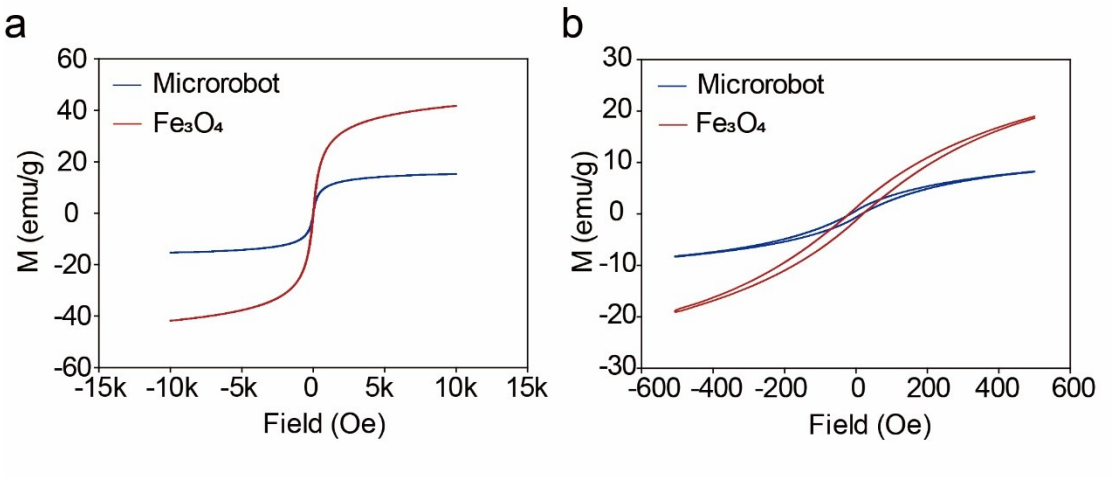


**Fig. S8 Magnetic properties.** (a) Magnetic property of $Fe_3O_4$-loaded hydrogel robot and $Fe_3O_4$ particles were examined with a vibrating sample magnetometer (VSM, LakeShore7404) by cycling the applied field from -10 to +10 kOe (major hysteresis loop). (b) The minor hysteresis loop was obtained by cycling the applied field from – 500 to +500 Oe. The test was at room temperature.

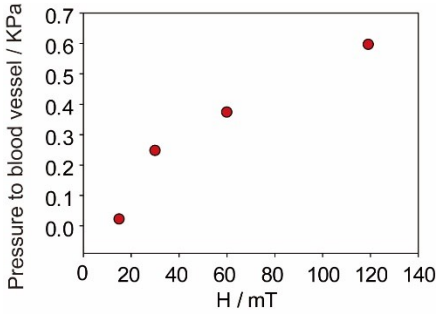


**Fig. S9** Evaluation of the pressure to the blood vessel. To evaluate the pressure caused by motion of the robot inside the blood vessels, we assumed that the pressure from the robot had completely applied to the blood vessel. In this case, the pressure could be calculated as formulas: $\Delta Vm = Ft$ and $P = F/S$, where $\Delta V$ was the average motion velocity (m/s) of the robot, m was the weight of the robot, t was the motion time, P was the pressure applied to blood vessel, and S was the cross-section area of the tubular robot. The formulas were derived from the Laws of motion reported by De Gosson, Maurice. Principles of Newtonian and Quantum Mechanics. River Edge, NJ: World Scientific Publishers, 2001.

The maximum pressure caused by the motion of the robot driven by magnetic intensity of 120 mT was 0.6 KPa that was not enough to destroy blood vessels, since the blood vessels are normally able to support a pressure of ~15.9 KPa that was calculated as the supportable blood pressure of 120 mmHg

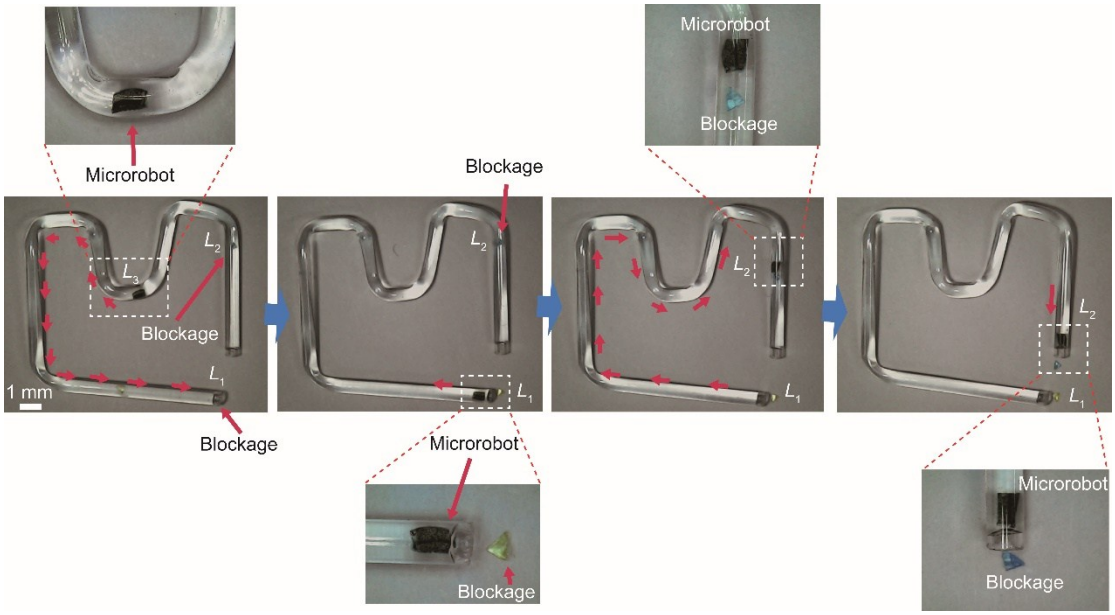


**Fig. S10** Motion of a hollow hydrogel microrobot along a circuitous tubular channel. A circuitous capillary tube filled with water was used to mimic a segment of non-linear artificial blood vessel. The microrobot was driven for directional motion by external magnetic actuation. Its initial position was at $L_3$ inside the capillary tube. It moved along the channel and first reached $L_1$ position where an artificial blockage was located. After the blockage at $L_1$ was removed out, the microrobot shifted back to the second blockage at $L_2$ and clear up it.