Supporting Information

Utilizing Photosynthetic Oxygen-Releasing Biomaterials to Modulate Blood Vessel Growth in Chick Embryo Chorioallantoic Membrane

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1 · Cyanobacteria treatment

Synechocystis sp (pcc6803), belonging to the phylum Cyanobacteria, was purchased from ATCC, U.S.A. Synechocystis sp. was spherical in shape, with an average diameter of 5-10 μm, and was inoculated into BG-11 medium (1.7 g of medium per litre of deionized water, sterilized at high temperature and autoclave for use). The culture was put into a shaker incubator for continuous cultivation under the following conditions: 100 rpm, 37℃, and the light source was provided by a full-spectrum lamp, and the whole process was carried out under aseptic conditions, and the experiment was started when the cyanobacteria entered into the logarithmic growth period. The specific operation steps were as follows:

Step 1: Selecting suitable cyanobacterial species

Before carrying out cyanobacterial culture, it is first necessary to select suitable cyanobacterial strains for culture. Commonly used cyanobacterial strains include Spirulina, Microcystis, Anabaena and so on. Different species of cyanobacteria have different culture conditions and uses, so it is important to choose the appropriate strain according to specific needs. The species chosen for use in this experiment is pcc6803.

Step 2: Preparation of culture medium

The culture medium of cyanobacteria is one of the crucial factors in the cultivation process. The formula of culture medium usually includes carbon source, nitrogen source, phosphorus source, trace elements and so on. Commonly used culture media for cyanobacteria include BG-11 medium, Z8 medium and so on. In the process of preparing the culture medium, it is necessary to make sure that the ratio and concentration of each component is in accordance with the needs of the strain. In this experiment, BG-11 medium was used for preparation, and the concentration of preparation was 1.7g of culture medium powder per litre of deionized water dissolved and then subjected to high-temperature and high-pressure disinfection, and then cooled down on the UV-disinfected operating table before being prepared for use.

Step 3: Prepare culture container and culture conditions

Choosing suitable culture containers and culture conditions is crucial for

cyanobacteria cultivation. Usually, transparent culture flasks or conical flasks can be chosen, and ensure that the culture container has proper aeration. The culture of cyanobacteria requires certain light and temperature conditions, usually providing 12 hours of light and 12 hours of darkness at a temperature of 25-30℃. In this experiment, 250 mL conical flasks were used, together with high-temperature-resistant and breathable sealing film (convenient for high-temperature and high-pressure disinfection), and the whole was put into a light incubator for the regulation of ambient temperature and light, and the temperature was controlled to be at 28±0.5℃.

Step 4: Inoculate the culture medium

Inoculation is the key step to start cyanobacterial culture. Inoculation of cyanobacterial strains into the culture medium can be carried out by direct inoculation or pre-treatment inoculation. Aseptic operation needs to be paid attention to during inoculation in order to avoid the influence of exogenous contamination on the culture. In this experiment, pcc6803 algal concentrate was inoculated directly into the configured medium.

Step 5: Culture process management

During the culture process of cyanobacteria, the state of the culture medium needs to be checked regularly to maintain proper culture conditions. Parameters such as pH value, dissolved oxygen and temperature of the culture medium need to be monitored and the culture conditions adjusted as needed. Attention also needs to be paid to prevent the occurrence of exogenous pollution and keep the culture environment clean. In this experiment, the Hash HQ4300 fluorescent probe was used to track and measure the pH, dissolved oxygen, and temperature of the culture system in order to facilitate the adjustment of its optimal growth environment.

Step 6: Harvesting and processing

When the cyanobacteria grow to a certain density, they can be harvested and processed. In this experiment, a centrifuge at 3000 rpm was used to separate the cyanobacteria, and the concentrated cyanobacterial liquid was obtained after 20 min of treatment for use in subsequent experiments.

Step 7: Dispose of waste liquid

In the process of cyanobacteria cultivation, a certain amount of waste liquid will be produced, which needs to be treated reasonably. The cyanobacterial residues in the waste liquid can be removed by precipitation, clarification and filtration to reduce the impact on the environment. Environmental protection requirements need to be paid attention to when treating the waste liquid to avoid negative impacts on the surrounding environment. The waste liquid generated from this experiment is recycled and uniformly treated by professional hazardous waste treatment company.

Step 8: Analysis and evaluation after culture

After completing the cyanobacterial culture, the analysis and evaluation of cyanobacterial cells can be carried out. Observe the morphology, colour and other characteristics of the algal cells through a microscope, and also carry out experiments such as biochemical analysis and growth rate assessment to understand the growth and physiological state of the cyanobacteria.

Step 9: Maintain the culture medium and keep the strain

In order to cultivate cyanobacteria stably for a long period of time, we change the culture medium regularly every fortnight, keep the culture container clean, and check the purity and activity of the strains regularly. Maintaining the culture medium and keeping the strain alive will ensure the long-term stable culture of cyanobacteria.

Step 10: Optimize culture conditions and increase yield

In the process of cyanobacteria cultivation, 0.5 mL of sodium bicarbonate solution with a concentration of 1 mol/L was added dropwise to supplement the carbon source after adding the same volume of culture solution each time after taking, through which the culture conditions and cultivation techniques were continuously optimized to improve the yield and quality of cyanobacteria. Parameters such as light intensity, temperature and oxygen supply can also be adjusted to improve the growth rate and yield of cyanobacteria.

2. Preparation of oxygen-releasing hydrogel microparticles with cyanobacteria

Preparation of cyanobacterial concentrate: first, the cyanobacterial suspension was incubated and centrifuged at 3000 rpm for 20 min to remove the supernatant.

Preparation of cyanobacteria-loaded dispersed phase: the cyanobacteria solution was mixed with sodium alginate solution as wall material in a specified ratio and stirred well to obtain the core solution.

Preparation of microdroplets by interfacial shear: using a 10 mL syringe as a container, the core solution was aspirated and placed in a high-precision syringe pump, and the flow rate was adjusted. Turn on the signal generator, so that the needle at the gas-liquid interface for cyclic shear movement, the dispersed phase will be dispersed into micro droplets.

Curing into hydrogel microparticles: Drop by drop, the microdroplets are dropped into calcium chloride solution, which solidifies the micro droplets into microparticles through the cross-linking effect of sodium alginate and calcium chloride solution.

Washing and centrifugation: After completing all the solution shearing, centrifuge at 300 rpm for 15 min, remove the supernatant, and wash with a small amount of deionized water for 2-3 times to obtain the microparticles.

Vacuum freeze-drying: the microparticles were vacuum freeze-dried for subsequent use.

In this paper, a one-way experimental method was used to optimize the process of cyanobacterial hydrogel microparticles, and five factors affecting the cyanobacterial hydrogel microparticles were selected, namely, the mass fraction of sodium alginate, the mass fraction of CaCl2, the volume ratio of cyanobacterial concentrate to sodium alginate, the frequency of interfacial shear, and the injection flow rate, and each of these factors was subjected to five different level variables, with the following specific conditions: the mass fractions of sodium alginate: 1 %, 1.5%, 2%, 2.5%, 3%; CaCl2 mass fraction: 0.1%, 0.3%, 0.5%, 0.7%, 0.9%; cyanobacteria to sodium alginate volume ratios of 1:1, 1:3, 1:5, 1:7, 1:9; interfacial shear frequency: 1, 2, 3, 4, 5 Hz; and injection flow rates of 11, 13, 15, 17, and 19 mL/h. In each group of factors the At different levels, the experiments were carried out separately to determine the effects of each group of factors on the morphological characteristics and particle size changes of the cyanobacterial hydrogel microparticles as the indicators for evaluating their performance, so as to carry out a preliminary exploration and optimization of the

optimal preparation process conditions of the cyanobacterial hydrogel microparticles. In the single-factor experiments, when studying the effect of one factor on the cyanobacterial hydrogel microparticles, all other factors were kept under the optimal conditions for experimental operation, such as when exploring the effect of sodium alginate mass fraction on the cyanobacterial hydrogel microparticles, then all other conditions were fixed as follows: $CaCl_2$ concentration of 0.5%, the core-to-wall ratio of 1:5, the frequency of 3 Hz, and the injection flow rate of 15 mL/h for the single-factor experiments.

Finally, the wrapped hydrogel microparticles were placed in a petri dish, and certain deionized water was dripped in to make them dispersed, and they were observed with a stereomicroscope at 0.75 magnification, photographed, their image files were saved, and the diameter size of the hydrogel microparticles was measured using an image-processing program, and their data were recorded. Using five-point sampling method, 100 hydrogel microparticles were randomly taken for diameter measurement to get their mean value and distribution interval, and finally, Origin was used for statistical analysis and graphing to show the changes in the particle size distribution of hydrogel microparticles.

2.1 Reagent concentration affects microparticle morphology

Effect of sodium alginate concentration. Some relevant studies have shown that the concentration of sodium alginate in the prepared wall solution should not be too high, otherwise the embedding effect will be affected. Although the high viscosity of the condensed phase is conducive to the production of more stable hydrogel microparticles, if the viscosity is too high, the morphology and particle size of the hydrogel microparticles will change, and the embedding effect will be weakened instead.

In order to verify this point, different concentrations of sodium alginate solution were used to prepare cyanobacterial hydrogel microparticles in this paper, and their morphology and particle size were observed. As shown in **Fig. S1(a-e)**, the morphology of the cyanobacterial hydrogel microparticles gradually became rounded with the increase of sodium alginate concentration, and the best morphology was achieved at 2%, and then gradually began to become irregular with the further increase of the

concentration, and once again appeared to be more obvious trailing phenomenon at 3%. This may be due to the fact that when the concentration of sodium alginate is too low or too high, it will affect the cross-linking reaction between sodium alginate and calcium chloride, resulting in incomplete or oversized hydrogel microparticle formation. As shown in **Fig. S1(f)**, the average particle size of cyanobacterial hydrogel microparticles increased gradually with the increase of sodium alginate concentration and levelled off; it may be because the concentration of sodium alginate is 1%, the concentration is low, which can not be rapidly cross-linked to form a sphere when dropping into the calcium chloride solution, and the flow pump is constantly pushing the cyanobacteria-sodium alginate mixture solution into the calcium chloride, which leads to the phenomenon of dragging the tail, and when the concentration of sodium alginate is 3% , because the concentration is too high, the amount of cross-links formed also increases, thus making the hydrogel microparticles larger in size, because the pore size of the needle remains the same, so the final formation of irregular spherical shape.

For comprehensive consideration, the concentration of sodium alginate of 2% is more suitable for the preparation of cyanobacterial hydrogel microparticles, because the morphology of the hydrogel microparticles under this condition is more regular, and the wall membrane is rounded and smooth, with no obvious trailing and adhesive state.

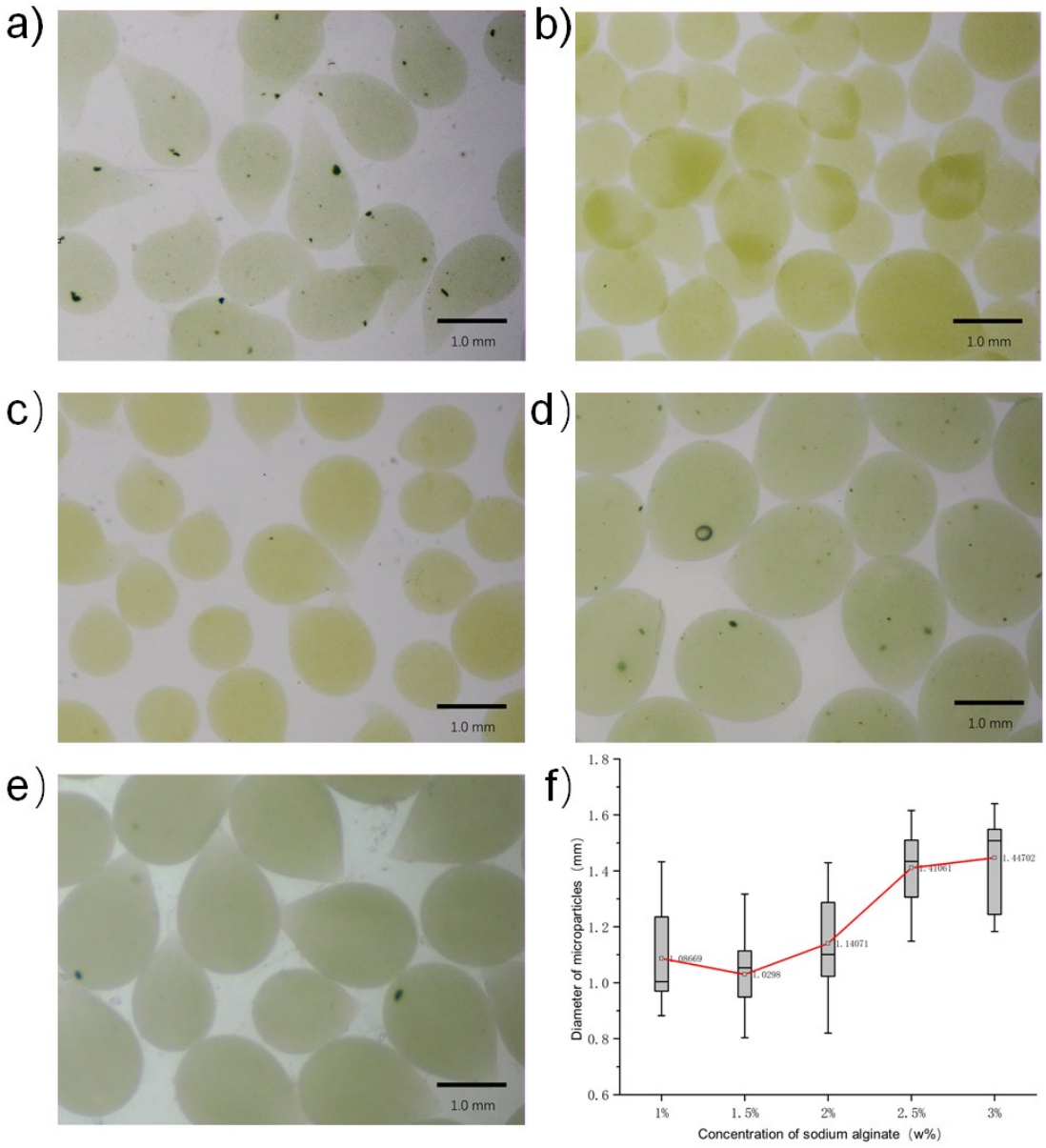

Fig. S1 Effect of sodium alginate concentration on the morphology of cyanobacterial hydrogel-loaded microparticles. (a) Cyanobacterial microparticles prepared at 1% sodium alginate concentration as a variable; (b) Cyanobacterial microparticles prepared at 1.5% sodium alginate concentration as a variable; (c) Cyanobacterial microparticles prepared at 2% sodium alginate concentration as a variable; (d) Cyanobacterial microparticles prepared at 2.5% sodium alginate concentration as a variable; (e) Cyanobacterial microparticles prepared at 3% sodium alginate concentration as a variable. (f) Statistical graph of the effect of sodium alginate concentration on the particle size of cyanobacterial hydrogel-loaded microparticles.

Effect of calcium chloride concentration on particle size. The degree of structural looseness of hydrogel microparticles has a key role in the morphology and particle size

of hydrogel microparticles, so the concentration of calcium ions has a certain effect on the degree of denseness of the structure of hydrogel microparticles and mechanical strength.

As shown in **Fig. S2(a-e)**, with the increase of calcium chloride concentration, the cyanobacterial hydrogel microparticles from the beginning at 0.1% will appear trailing phenomenon, to the morphology is gradually regular, in the form of a sphere, and the average volume of the first decrease followed by an increase in the change. This is because initially the concentration of calcium chloride is low, the calcium ions in the solution is insufficient, can not make the liquid under the action of shear force quickly into a ball, droplets crosslinked in the water for a period of time before becoming a ball, hydrogel microparticles loose structure eventually become droplet-shaped, and the volume of a larger; with the increase in the concentration of calcium ions is sufficient, and can be combined with alginate root ions quickly to form hydrogel microparticles crosslinking points, the structure of a dense, the volume of a smaller; as shown in **Fig. S2(f)**, with further increase in concentration, calcium ions were in excess relative to sodium alginate, and more aggregates were formed by cross-linking, which ultimately led to an increase in the volume of cyanobacterial hydrogel microparticles.

Therefore, when preparing the cyanobacterial hydrogel microparticles, the concentration of calcium chloride is 0.5% which is more appropriate, at this time, the morphology of the cyanobacterial hydrogel microparticles is regular, and the size of the particles is moderate.

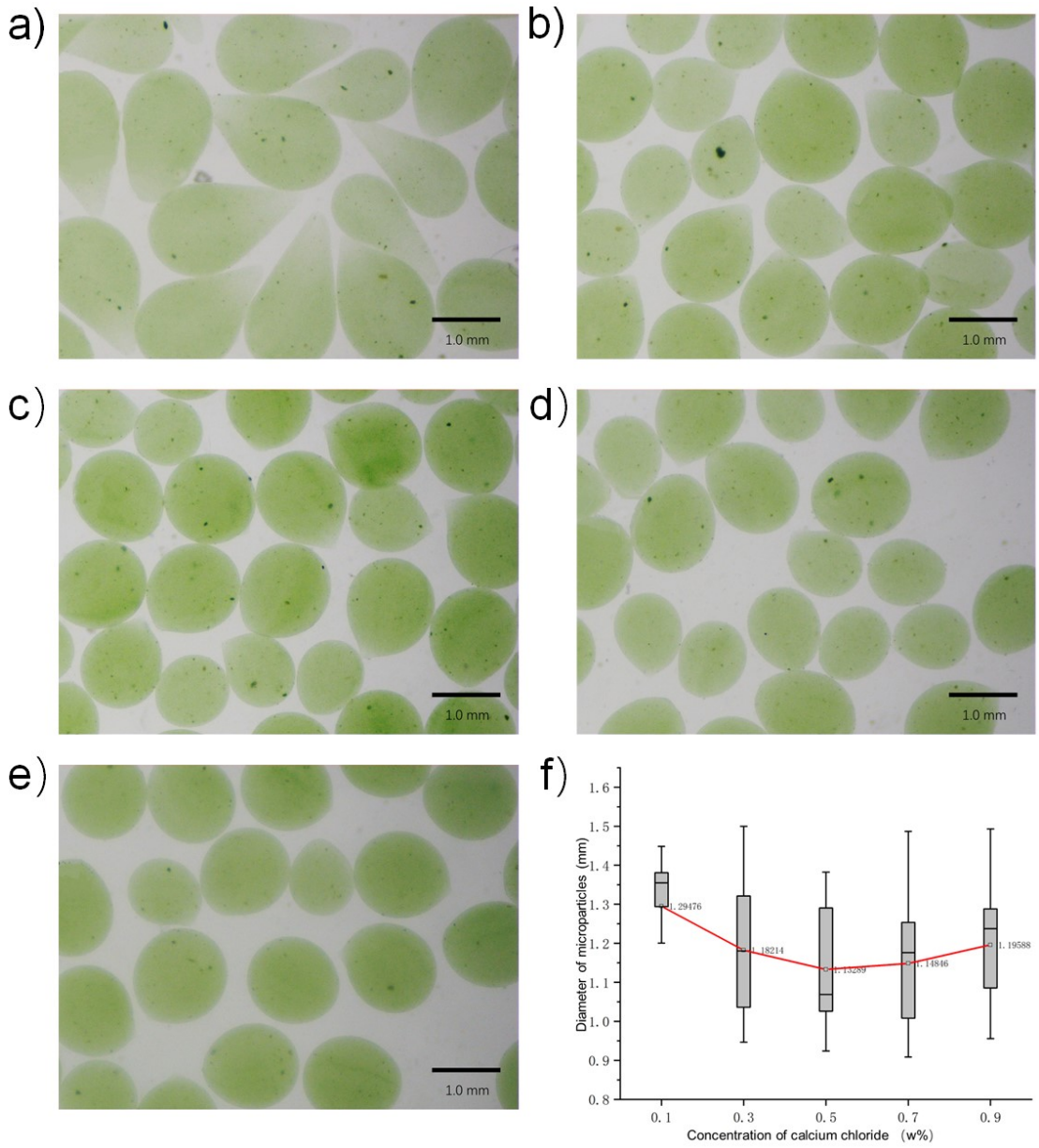

Fig. S2 Effect of calcium chloride concentration on the morphology of cyanobacteria-loaded hydrogel microparticles. (a) Cyanobacterial microparticles prepared at 0.1% calcium chloride concentration; (b) Cyanobacterial microparticles prepared at 0.3% calcium chloride concentration; (c) Cyanobacterial microparticles prepared at 0.5% calcium chloride concentration; (d) Cyanobacterial microparticles prepared at 0.7% calcium chloride concentration as a variable; (e) Cyanobacterial microparticles prepared at 0.9% calcium chloride concentration as a variable; (f) Statistical graph of the effect of calcium chloride concentration on the particle size of cyanobacteria-loaded hydrogel microparticles statistical plot

2.2 Relative flow rate of dispersed phase affects microparticle morphology

In order to study the effect of injection flow rate on the morphology and particle size

of cyanobacterial hydrogel microparticles, we changed the gradient injection flow rate under the condition of fixed shear frequency of 2 Hz, and observed the morphology and particle size of hydrogel microparticles. The results are shown in **Fig. S3(a-e)**, the cyanobacterial hydrogel microparticles with each gradient injection flow rate can form a better spherical structure, which shows that the injection flow rate has little effect on the morphology of hydrogel microparticles. **Fig S3(f)** shows the experiments carried out under the condition of fixed shear frequency of 2 Hz, with Q changing from 11 mL/h to 19 mL/h, the average diameter of hydrogel microparticles increased from 1.0142 mm by 1.2689 mm, which is an increase of about 25%.

Considering the morphology particle size of cyanobacterial hydrogel microparticles and time cost, the injection flow rate of 13 mL/h was selected for the preparation of cyanobacterial hydrogel microparticles by interfacial shear technique, when the morphology of hydrogel microparticles was regular and the particle size distribution was uniform.

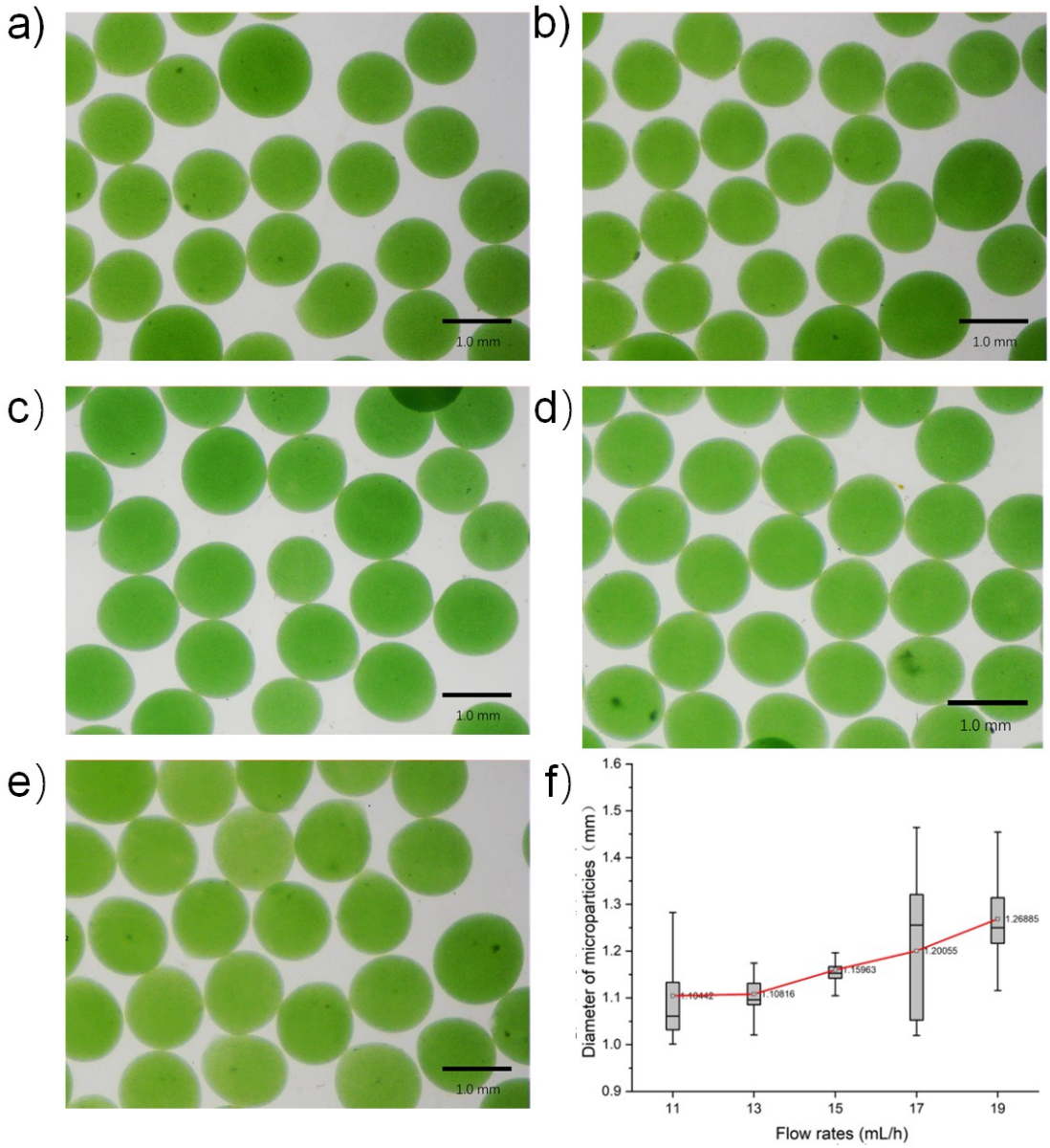

Fig. S3 Effect of relative flow rate of dispersed phase on the morphology of cyanobacterial hydrogel-loaded microparticles. (a) Cyanobacterial microparticles prepared at a relative flow rate of 11 mL/h for a single variable dispersed phase; (b) Cyanobacterial microparticles prepared at a relative flow rate of 13 mL/h for a single variable dispersed phase; (c) Cyanobacterial microparticles prepared at a relative flow rate of 15 mL/h for a single variable dispersed phase; (d) Cyanobacterial microparticles prepared at a relative flow rate of 17 mL/h for a single variable dispersed phase; (e) Cyanobacterial microparticles prepared at a relative flow rate of 19 mL/h for a single variable (e) Cyanobacterial microparticles prepared at a relative flow rate of 19 mL/h of the dispersed phase; (f) Statistical graph of the effect of relative flow rate of the dispersed phase on the size of microparticles of cyanobacterial-loaded hydrogels

2.3 Shear frequency affects microparticle morphology

As an emerging microdroplet active generation technique, the microdroplet generation frequency of interfacial shear technique is roughly equal to the vibration frequency of the needle, and when the conditions of the wall material and cross-linking agent concentration are determined, the size of the microdroplet is affected by two experimental parameters, namely, the flow rate of the dispersed phase and the vibration frequency of the syringe needle, and the theoretical volume of a single hydrogel microparticle is able to be expressed by the formula if the volume of gelatinisation is unchanged in the solution The volume of a droplet (V) is equal to the flow rate (Q) divided by the vibration frequency (f):$V=Q/f$, and the cyanobacterial hydrogel microparticles can be approximately regarded as spherical, so the volume formula can also be expressed by the following formula, i.e., $V=\frac{4}{3}\pi(\frac{D}{2})^3$, combining the above two formulas can be derived from the theory of the diameter of the cyanobacterial hydrogel microparticles and the shear frequency with the injection flow rate formula: $D=\left(\frac{6Q}{\pi f}\right)^{\frac{1}{3}}$.

As shown in **Fig. S4(a-e)**, with a fixed injection flow rate of 15 mL/h, as the frequency of interfacial shear gradually increases, the morphology of the cyanobacterial hydrogel microparticles changes from irregular ellipsoid shape to round shape, which is better for vesiculation. As shown in **Fig. S4(f)**, the particle size of hydrogel microparticles gradually decreased with the increase of shear frequency. It can also be found that at 3 Hz and 5 Hz, a larger range of particle size distribution occurs, which may be attributed to the influence of larger morphology of cyanobacterial hydrogel microparticles due to occasional loss of frequency during the working process.

Considering the morphology of hydrogel particles and particle size distribution, the shear frequency was chosen to be 2 Hz, at which time the morphology of hydrogel particles was regular and the particle size distribution was more uniform.

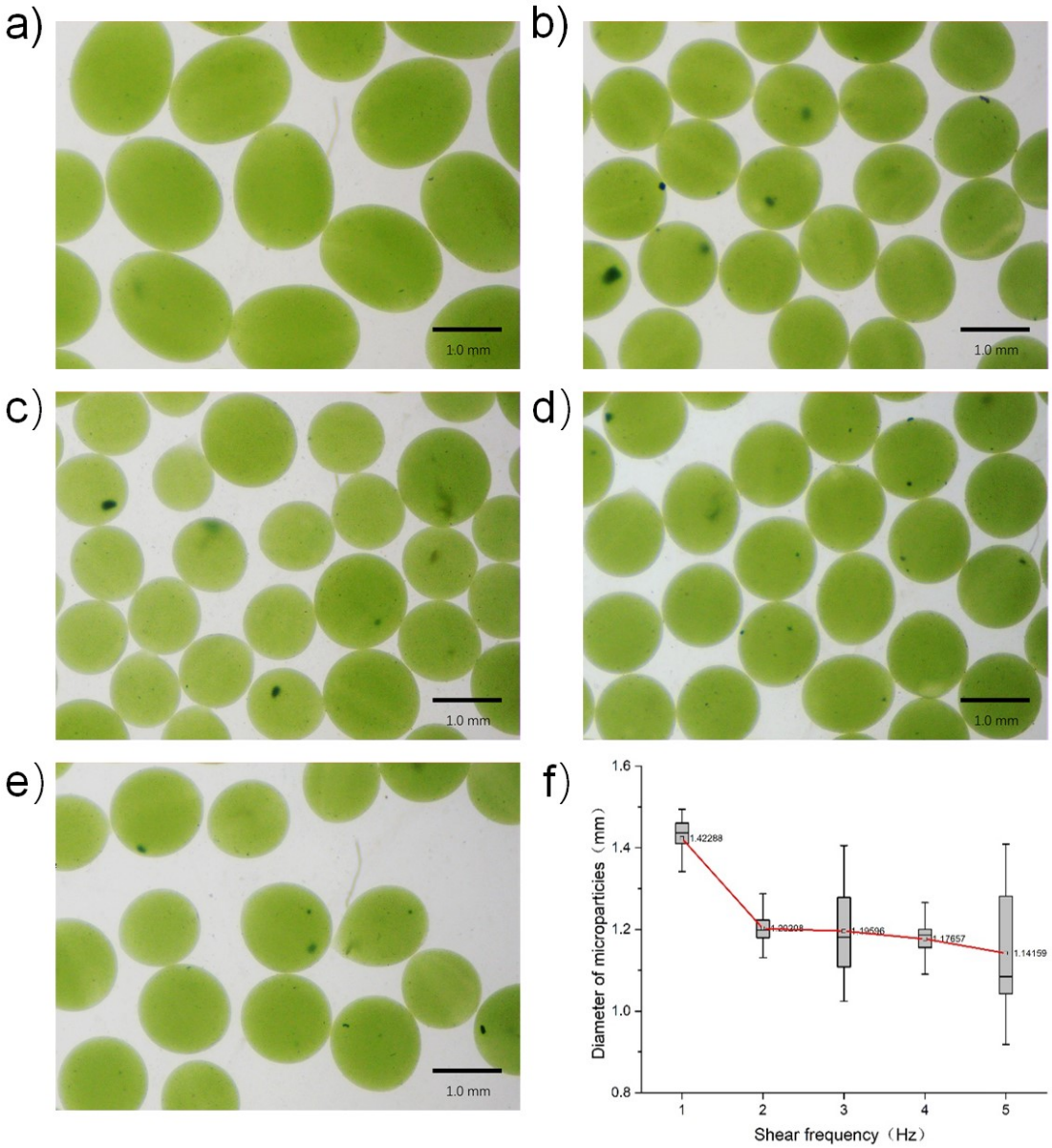

Fig. S4 Effect of shear frequency on the morphology of cyanobacterial hydrogel-loaded microparticles. (a) Cyanobacterial microparticles prepared at a single variable shear frequency of 1 Hz; (b) Cyanobacterial microparticles prepared at a single variable shear frequency of 2 Hz; (c) Cyanobacterial microparticles prepared at a single variable shear frequency of 3 Hz; (d) Cyanobacterial microparticles prepared at a single variable shear frequency of 4 Hz; (e) Cyanobacterial microparticles prepared at a single variable shear frequency of 5 Hz; (f) Statistical graph of the effect of shear frequency on the particle size of cyanobacterial-loaded hydrogel microparticles. (f) Statistical plot of the effect of shear frequency on the particle size of cyanobacterial hydrogel-loaded microparticles

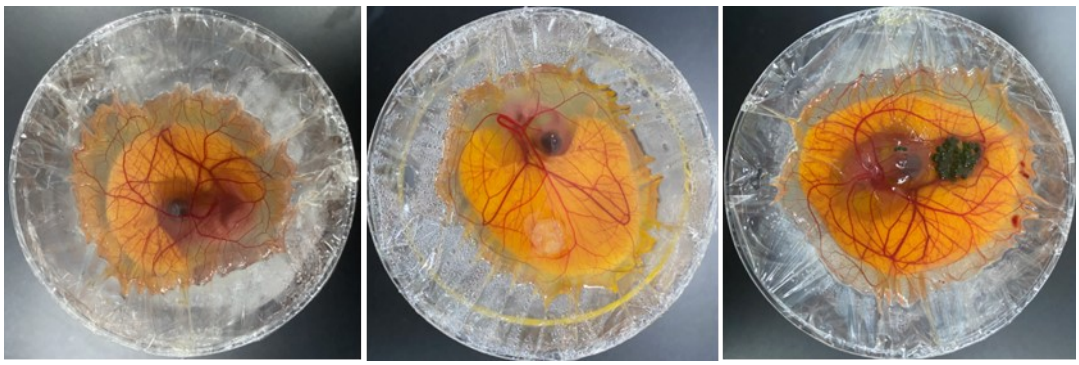

Fig. S5 Development of blood vessels in the presence of calcium alginate hydrogel microspheres containing cyanobacteria added to five groups of CAM.

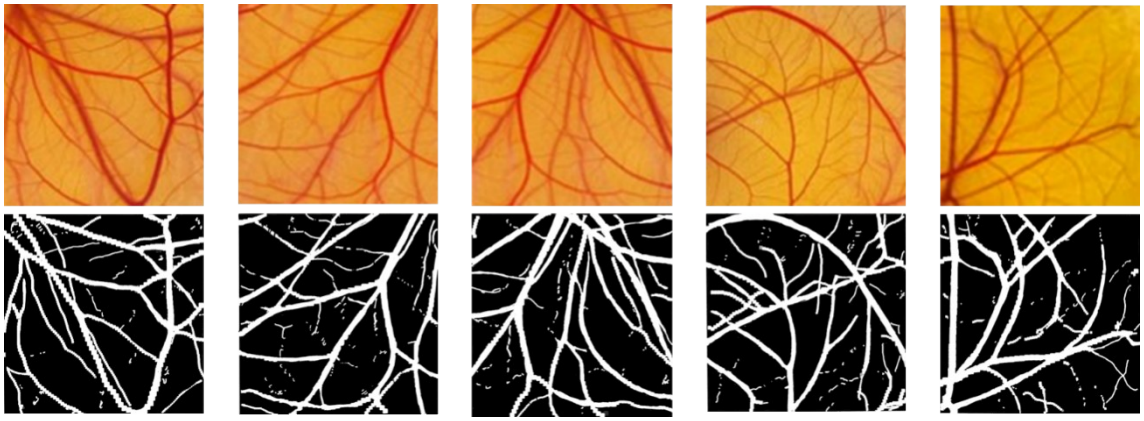

Fig. S6 Five sets of blank experiments on the development of vasculature in natural growth of CAM.

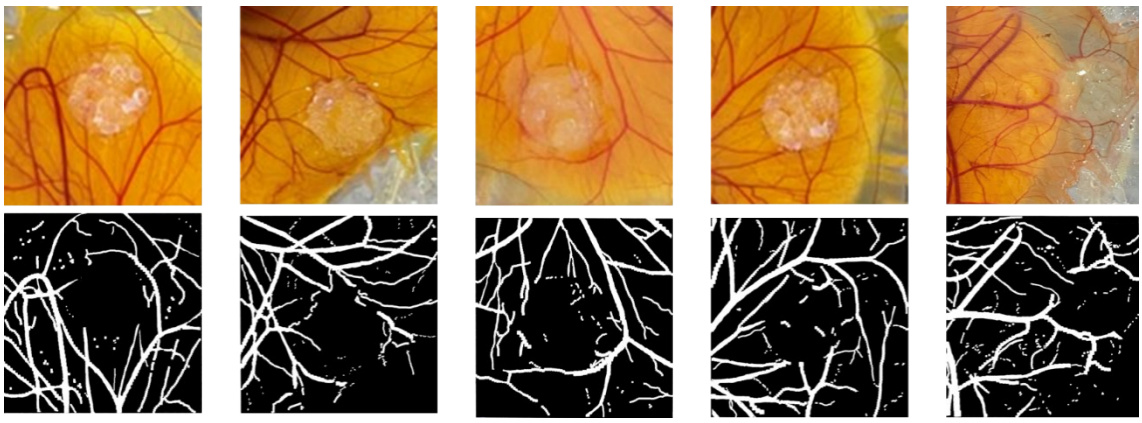

Fig. S7 Development of blood vessels with the addition of calcium alginate hydrogel microspheres on five groups of CAM.

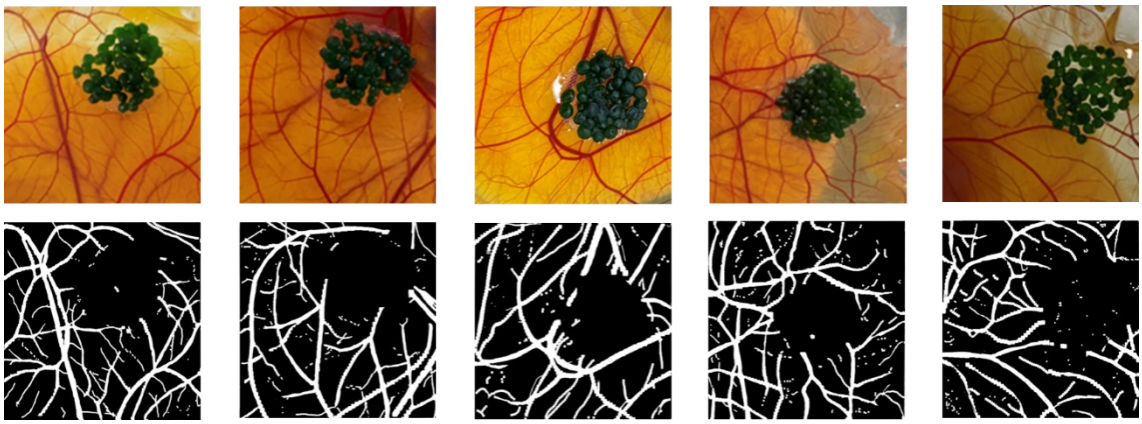

Fig. S8 Development of blood vessels in the presence of calcium alginate hydrogel microspheres containing cyanobacteria added to five groups of CAM.

Table. S1 Comparison data of vascular density and number of vessels in the CAM among the untreated group, calcium alginate blank group, and microalgae experimental group.

Group		1	2	3	4	5
Blank	Vascular density（%）	23.13	21.38	24.86	22.93	22.37
	Number of vessels	36	38	40	39	34
Gel	Vascular density（%）	23.67	21.30	20.53	23.85	23.37
	Number of vessels	40	38	41	41	42
Alg-gel	Vascular density（%）	25.07	25.53	27.32	24.89	22.86
	Number of vessels	62	60	62	66	60