Supporting Information

Polymerization induced phase separation as a generalized methodology for multi-layered hydrogel tubes

Mingming Rong[1,2], Shuanhong Ma[1], Peng Lin[3], Meirong Cai[1], Zijian Zheng[4], Feng Zhou[1*]*

1. M.M. Rong, Dr. S. H. Ma, Dr. M. R. Cai, Prof. W. M. Liu, Prof. F. Zhou
State Key Laboratory of Solid Lubrication, Lanzhou Institute of Chemical Physics, 18 Middle Tianshui Road, Lanzhou, China, 730000

2. M.M.Rong
University of the Chinese Academy of Sciences, Beijing, 100049, China

3. Dr. P. Lin
Anhui University of Technology, MaAnshan, 243032, China

4. Institute of Textiles and Clothing, the Hong Kong Polytechnic University, Hong Kong SAR, 999077, China

*E-mail: zhouf@licp.cas.cn; mashuanhong@licp.cas.cn;

Keywords: surface-initiated polymerization, hydrogel tubes, multi-layered, biocompatible, 3D complex structure

EXPERIMENTAL SECTION

1. Materials.

Sodium Alginate Medium viscosity (SA), acrylic acid (AAc), acrylamide(AAm),and N,N'-Methylenebis(acrylamide) (MBAA) were purchased from Sigma-Aldrich, and ammonium persulfate (APS), Calcium chloride anhydrous ($CaCl_2$) and iron chloride hexahydrate ($FeCl_3.6H_2O$) were purchased from Sinopharm, China.2-Hydroxyethyl methacrylate (HEMA) was purchased from J&K Chemical,China.

2. Fabrication of hollow bilayer tubular hydrogel with complex array.

The PHEMA/SA-Ca^{2+} tubular hydrogel was synthesized by the radical polymerization of HEMA monomers while the alginate sodium polymer chains interpenetrating among the first network. First, 2.5g SA powder was added into 50mL distilled water and heated to 60°Cwhile mixed by a magnetic bar until totally dissolved. Then, 6.5g HEMA(1mol·L^{-1}),0.01g MBAA(0.065mmol),0.02g APS(0.088mmol) and 100μL AAc was dripped into the solution with stirring violently to make the mixture homogeneously. After that the solution was put in the centrifuges (TG16-WS,CENCE, China) to remove bubbles. Then the iron wire(1.6mm) was immersed into the solution to proceed the polymerization for 15,30,45,60min at the room temperature, then they were moved into $CaCl_2$ solution(2.5%,5%,10%) to allow post-treatment for 2, 5 and 10h. At last, the iron wire can be removed. The concentration of SA can be various from 1% to 5% and HEMA varies from 0.5mol·L^{-1} to 3mol·L^{-1}. The kinetics of bilayer tubular hydrogel was investigated with 0.7mm iron wire. Correspondingly, the detailed amount of chemicals (HEMA, sodium alginate, BIS, APS, water) used for preparation of each hydrogel tube can be summarized in Table S1.

Table 1: The summary of material details for all bi-layer hydrogel tubes

No.	Polymerization time (min)	Sodium Alginate (%)	HEMA (mol·L^{-1})	Ca^{2+} (%)	APS (mmol)	BIS (mmol)	Water (mL)	AAc (μL)	Post-treatment time(h)
1	15	2.5	1	5	0.088	0.065	50	100	2
2	30	2.5	1	5					
3	45	2.5	1	5					
4	60	2.5	1	5					
5	45	1	1	5					
6	45	5	1	5					
7	30	2.5	0.5	5					
8	30	2.5	3	5					
9	45	2.5	1	2					
10	45	2.5	1	10					

11	30	1	1	5					
12	30	5	1	5					
13	30	2.5	1	2					
14	30	2.5	1	10					
15	30	2.5	1	5					5
16	30	2.5	1	5					10

The hydrogel arrays were prepared by the same procedure as above. By arranging the iron wire mold to attach with each other, the channels of array can be inter-connected to allow the liquid flowing freely. The mold used for mold was printed by a commercial printer.

3. Preparation of three-layered hydrogel tubes.

Multi-layered hydrogel tubes were synthesized by re-initiation and polymerization after bi-layered tube immersed in the prepared solution. 6.5g HEMA(1mol·L^{-1}), 0.01g BIS(0.065mmol),0.02g APS(0.088mmol) and 100μL AAc were dissolved in 50mL pure water with stirred until obtaining homogeneous solution. Then the as-prepared bi-layer hydrogel tubes PHEMA/SA were immersed in the mixture solution for 2h to observe soft and white substance wrapped around the original tubes. Then they were post-treated in Ca^{2+}(5%) for 2h to obtain three-layered PHEMA/SA-Ca^{2+}/PHEMA multi-layered hydrogel tubes.

The fabrication of three-layered hydrogel tube with AAm/AAc as the third layer was in the same procedure. 4.26g AAm, 0.432g AAc, 0.006g MBAA and 0.02g APS were dissolved in 50mL pure water and followed by the bi-layer tubes being immersed in it for 30min. Finally, the as-prepared hydrogel tubes were put into the Fe^{3+} 0.1mol·L^{-1} for 12h to obtain the multi-layer hydrogel tubes.

4. Mechanical testing of hydrogel tubes.

The mechanical stress-strain test was proceeded on an electrical universal material testing machine with a 500N load cell(EZ-Test, SHIMADZU) at a constant stretch velocity of 50mm min^{-1}. The tensile test was performed at room temperature after swelling equilibrium. The tensile stress is defined as:

$$\sigma = {}^{P}/_{A}$$

Where the P is the force loaded on the hydrogel and A is the calculated cross-section area of the tubular hydrogel. In this case, the A should be defined as:

$$A = \pi R^2 - \pi(R - r)$$

Where R is the outer radius of hydrogel tube while r means the radius of hollow part. Then the tensile strain is defined as:

$$\varepsilon = {}^{(L - L_0)}/_{L_0}$$

Where L is the longitude in the real time and L_0 is the original longitude of hydrogel tube before stretching.

To measure the mechanical strength of each layer separately, the bi-layered hydrogel tube was tested the holistic tensile strength at first. Next the outlayer of bilayer hydrogel tube was cut by the knife and peeled away piece by piece carefully to guarantee the integrity of inner layer. Then the tensile strength of inner layer was measured.

5. The morphology characterization.

The morphology of hydrogel tubes was obtained by the Scanning Electron Microscope JSM-5600LV at an accelerating voltage of 20kV. The composition of testing sample was 2.5%SA and 1M/L HEMA which proceeding polymerization for 30min and immersed in the 5% Ca^{2+} for 2h. The samples were frozen in the atmosphere of liquid nitrogen for 10min, then gotten dried in 1Pa for at 24h with freeze-drying method. The cross-section of hydrogel tube in swelling equilibrium situation was obtained by the optical microscope Olympus BX51. And 1mg.mL^{-1}Rhodamine 6G(J&K Chemical) in distilled water flowed through the arrays made of bilayer hydrogel tubes to enhance the interconnected channels of complex array. The fluorescent image was obtained from WFH-203B UV Analyzer.

6. The Composition Characterization.

The difference in composition between two layers was characterized with energy dispersive spectroscopy (EDS). And a Nicolet iS10 FT-IR spectrometer between 4000 cm^{-1} and 500 cm^{-1} was also used to record the FTIR spectra. The bilayer hydrogel tube should be separated into two parts and heated at 120°C until totally dry. Then they were grinded into powder to proceed the FT-IR test.

7. The Growth Kinetics Characterization.

A series of hydrogel tubes were prepared with the iron wire of 0.7mm to make sure the whole image can be obtained at the four times magnification with Olympus BX51 microscope. The side surfaces of hydrogel tubes were imaged under the microscope and then the diameters of whole tube were measured with arbitrary line from one side to other side. Then the thicknesses of respective inner and outer were obtained by measuring them on the cross-section image. The average number and error bar were collected when the amount of data was more than ten in every group.

8. The evaluation of bioactivity of hydrogel tubes.

8.1 Modification of Hydrogel.

The modification of hydrogel tube with Sulfo-SANPAH was followed the previous literature. The hydrogel tube was cut into two halves and sterilized with 70% ethanol for 30min and followed by washing three times with milli-Q water, then immersed the samples into water for 2h until swelling again. Next 1mg Sulfo-SANPAH dissolved in 1mL milli-Q. And 30μL of it was added on the hydrogel by followed exposure under UV irradiation for 10min and washed with PBS. The procedure was repeated for three times and washed residual solution away with PBS. After drying the sample with

clean nitrogen, the samples were immersed into 50μg.mL-1 collagen for 4h to improve cells adhesion.

8.2. Cell culture and maintenance.

NIH3T3 and WI38 cells were obtained from ATCC and the Hubrecht Institute in the Netherlands respectively and both were cultured in the low-glucose Dulbecco's Modified Eagle Medium(DMEM;Gibco), meanwhile Pulmonary Artery Endothelial Cells (PAEC) were from Lena Welsh at Uppsala University Sweden and cultured in DMEM/Ham's F-12 medium (DF;Gibco). The culture mediums were all supplemented with 10% fetal bovine serum (FBS;Gibco) ,1% L-glutamine and 1% penicillin-streptomycin(P/S,Thermo Fisher Scientific). The cells all were cultured at 37 °Cin 5% CO_2 atmosphere. After proceeding the procedure of cells detachment, 10μL of cells suspension at a density of $1*10^6$ per mL was dripped on the inner surfaces of hydrogel tubes. The culture medium was changed every two days.

8.3. Cell Morphology.

Immunofluorescent staining was processed to image the cells skeleton. After incubation on the surface of hydrogel tubes for 2d, the cells were fixed with 4% paraformaldehyde (Sigma) for 10min followed by washed twice in DPBS. Then treated with 0.2% Triton X-100 (Sigma) for another 10 min at room temperature. After washed with DPBS for twice, the samples were immersed into DPBS solution mixed with phalloidin-Atto 633 (volume ratio:500;Sigma) and 4',6-diamidino-2-phenylindole(DAPI) (1:500;Millipore) for 1h.

For image the collagen coated on the surfaces of hydrogel, the fix of collagen was followed by staining firstly the collagen protein with Mouse antibody collagen I (1:500; Abcam, ab90395) for 1h. Then, the Goat anti-mouse lgG 488(1:500; Invitrogen) was specifically bound with primary antibody for another 1h. The stained cells and collagen protein were all imaged with the Confocal Microscope SP8 and ImageJ software was used to assembly composite graphics.

8.4. Cell Proliferation.

The 3-[4,5-Dimethylthiazol-2—yl]-2,5-diphenyl-tetrazolium bromide (Sigma) assay was used to detect the cells proliferation at predetermined time point such as 1,3,5 and 7days. At each specific time point, the culture medium was removed, followed by 200 μL culture medium and 20μL 5mg.mL^{-1} MTT in DPBS were added into each well. Subsequently, the plate was incubated in 37°C, 5% CO2 for 4h. Thereafter the mixture was removed and 200μL DMSO was added instead. The plate was put on the oscillator for 20min to dissolve crystals completely. At last the solution was transferred into 96 well plate and the optical density value (OD) was determined under 550nm wavelength with TriStar2 Multimode Reader LB942(Berthold Technologies).

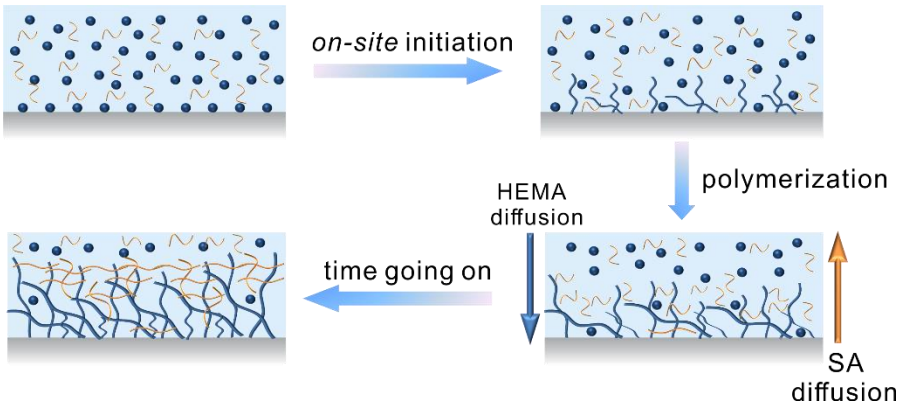

Figure S1: The schematic diagram showing the detailed polymerization mechanism to form layered/gradient PHEMA/SA hydrogel network on the surface of the iron wire in homogeneous HEMA/SA mixed solution.

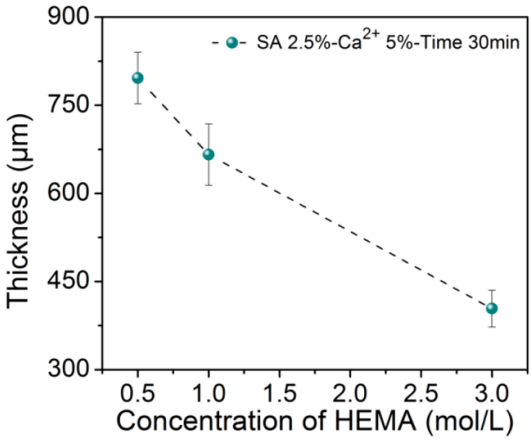

Figure S2: The effect of HEMA concentration in monomer solution on the thickness of holistic tube wall (SA: 2.5%, Ca^{2+}: 5% and polymerization time: 30 min). (The wall thickness of the whole tube decreases obviously with the HEMA monomer concentration increases. This concentration-related growth phenomenon seems violating the traditional solution polymerization kinetic. One possible reason is that HEMA monomers with high concentration would couple with SA polymer chains and cause the viscosity of the pre-polymerization solution significantly increasing, which could suppress effective diffusion of HEMA monomers in the polymerization process. As a result, low concentration of HEMA monomers is beneficial to grow layered hydrogel tubes with thicker walls.)

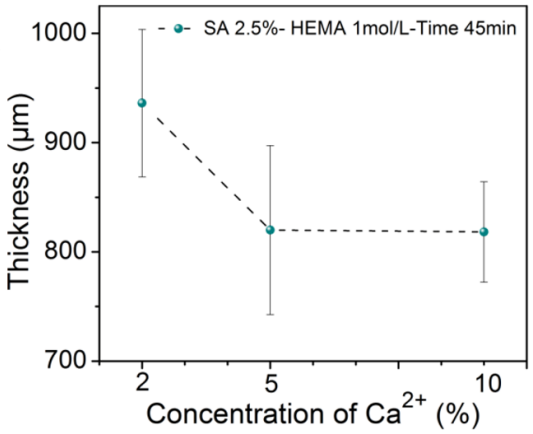

Figure S3: The effect of Ca^{2+} concentration in post-treatment process on the thickness of the holistic tube wall SA: 2.5%, HEMA: 1 mol·L^{-1} and polymerization time: 45 min).All the post-treatment time in $CaCl_2$ solution was 2 h. (In a typical case, raising the Ca^{2+} concentration is favorable for obtaining a denser SA-Ca^{2+} physical cross-linking network in hydrogel tube and results in obvious volume shrinkage of the tube wall thickness. When the Ca^{2+} concentration reaching to 5%, the coordination degree between Ca^{2+} and SA is up to balance. Importantly, the results above are in good agreement with the suggested gradient forming mechanism of layered hydrogel tube that outer layer is dominated by SA-Ca^{2+} physical cross-linking network while the inner layer is mainly composed by the PHEMA chemical cross-linking network.)

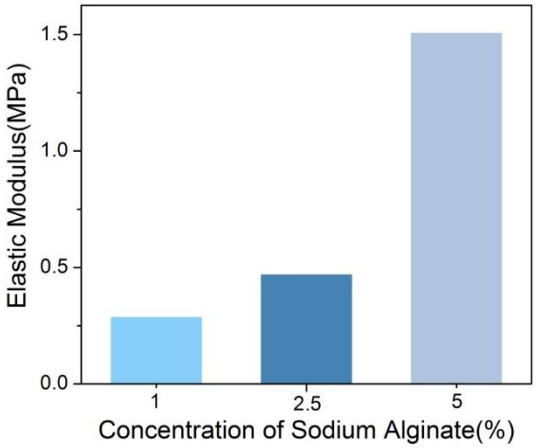

Figure S4: Elastic modulus vs concentration of sodium alginate of PHEMA/SA-Ca^{2+} bilayer hydrogel tube. (Polymerization time: 30 mins, concentration of HEMA:1 mol·L^{-1}, Ca^{2+} 5%, post-treatment-time 2 h).

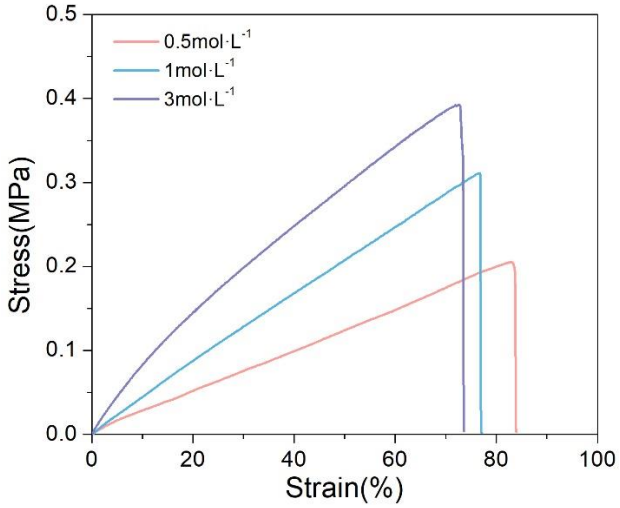

Figure S5: The stress-strain curves for the as-prepared bilayer hydrogel tubes by changing the HEMA concentration in monomer solution (polymerization time: 30 min, concentration of SA: 2.5%, Ca^{2+}: 5%, post-treatment time: 2 h)

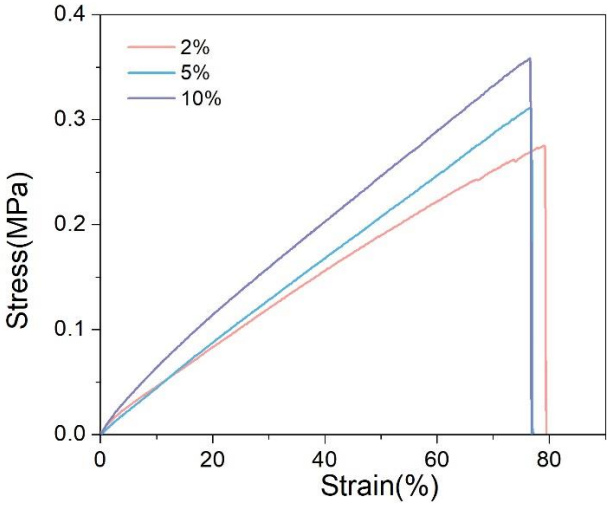

Figure S6: The stress-strain curves for the as-prepared bilayer hydrogel tubes by changing the concentration of Ca^{2+} in post-treatment process (polymerization time: 30 min, concentration of SA: 2.5%, HEMA: 1 mol·L^{-1}, post-treatment time: 2 h).

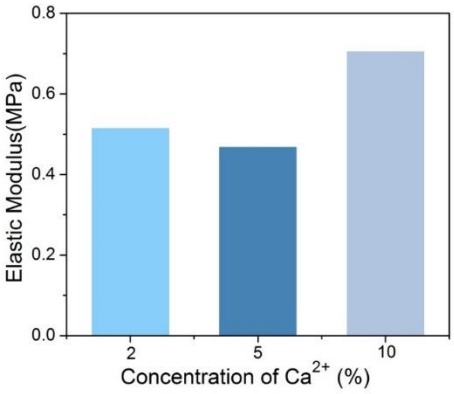

Figure S7: Elastic modulus vs concentration of Ca^{2+} in the process to prepare PHEMA/SA-Ca^{2+} bilayer hydrogel tube. (polymerization time:30min, concentration of HEMA:1 mol·L^{-1}, SA: 2.5%, post-treatment-time 2h).

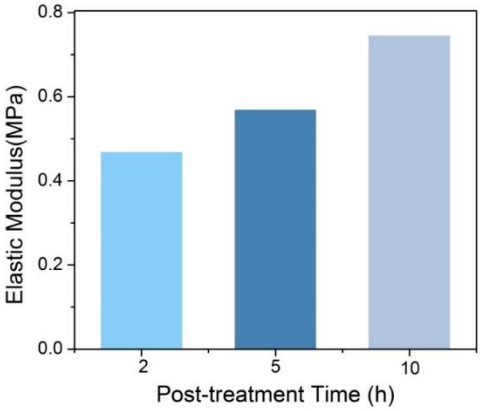

Figure S8: Elastic modulus vs post treatment timein $CaCl_2$ solution of PHEMA/SA-Ca^{2+} bilayer hydrogel tube. (polymerization time: 30min, concentration of HEMA:1 mol·L^{-1}, SA: 2.5%, Ca^{2+}: 5%).

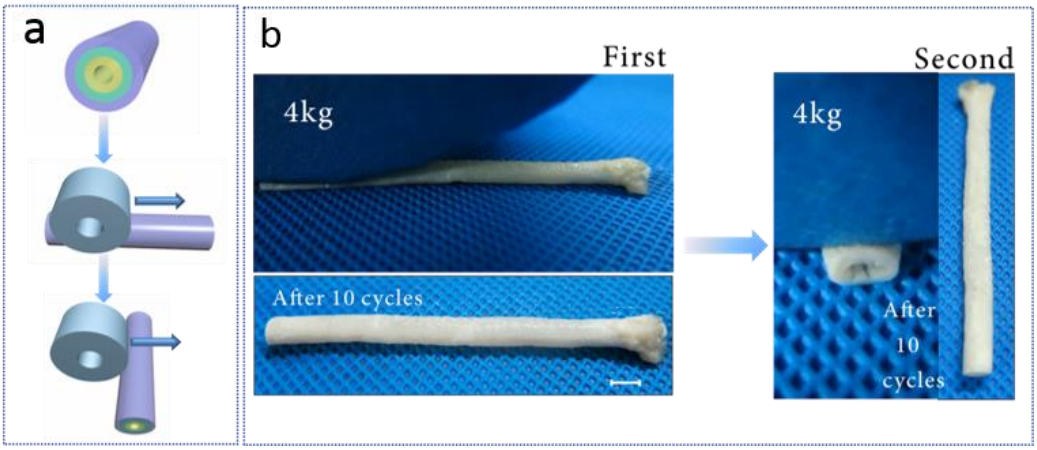

Figure S9: Compress test for the as-prepared PHEMA/SA-Ca^{2+} three-layered hydrogel tube. (a) The scheme of compress test for the as-prepared three-layered

hydrogel tube; (b) Sliding along the axial direction of the tube for ten times at first and then sliding along the radial direction for another ten times, by employing a 4kg steel block (Scale bar: 5mm)

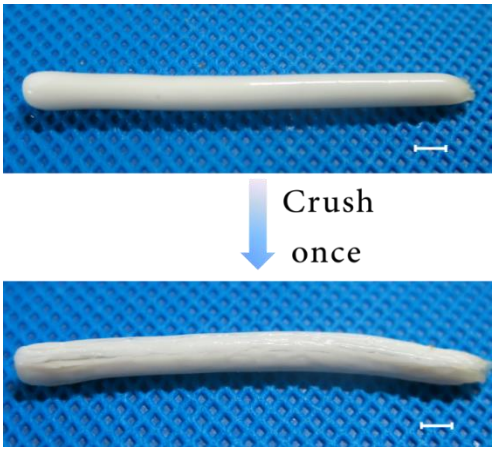

Figure S10: The photos of before and after the block crushing bilayer PHEMA/SA-Ca^{2+} hydrogel tube for only once respectively.(Scale bar: 5mm)

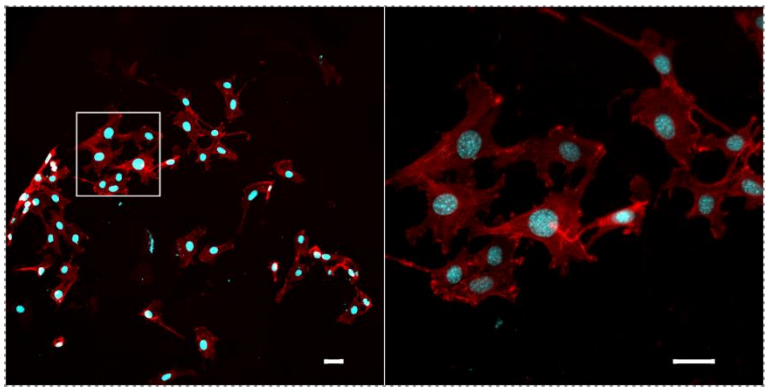

Figure S11: The fluorescent image showing NIH3T3 cells morphology which incubated on the inner surfaces of PHEMA/SA-Ca^{2+} hydrogel tube for 40h. (Scale bar: 30 μm)

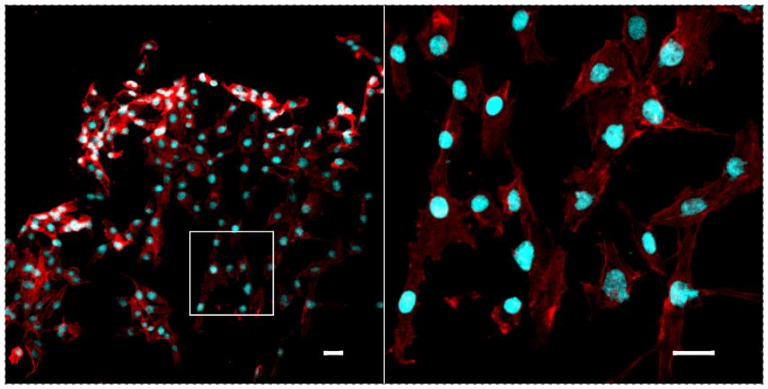

Figure S12: The fluorescent image showing PAEC cells morphology which incubated on the inner surfaces of PHEMA/SA-Ca^{2+} bilayer hydrogel tube for 40h. (Scale bar: 30 μm)

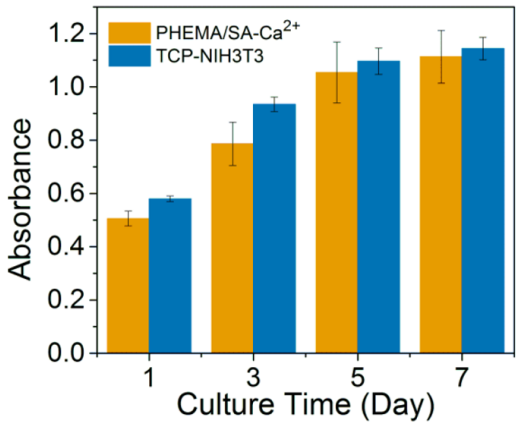

Figure S13: The biocompatibility of bilayer PHEMA/SA-Ca^{2+} hydrogel tube (the proliferation assay of NIH3T3 cells incubated on the inner surfaces of PHEMA/SA-Ca^{2+} hydrogel tube for one-week.)

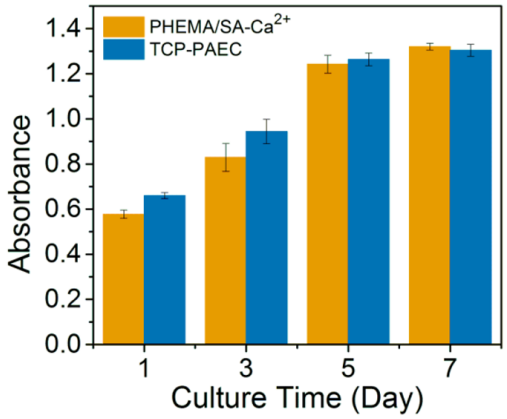

Figure S14: The biocompatibility of bilayer PHEMA/SA-Ca^{2+} hydrogel tube (the proliferation assay of PAEC cells incubated on the inner surfaces of PHEMA/SA-Ca^{2+} hydrogel tube for one-week.)