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Supplementary Information

Long-Lived Protein Expression in Hydrogel Particles: Towards Artificial Cells

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Experimental Procedures

Reagents

The PURE system kit (E3313), E.coli RNA polymerase (M0550S) and ribosome (P0763) were purchased from New England Biolabs. Poly(ethylene glycol) 600 (cat. no. 202401), AB-NTA (cat. no. 113231-05-3), acrylamide (cat. no. A3353), N, N'-Methylene bisacrylamide (cat. no. M7279), 2-hydroxy-2-methylpropiophenone (cat. no. 405655), acrylic acid N-hydroxysuccinimide ester (cat. no. A8060) were purchased from Sigma-Aldrich.

The feeding buffer contained 0.3 mM 20 amino acids (Aladdin), 10 mg/mL 10-formyl-5,6,7,8-tetrahydrofolic acid (Sigma), 56 A260/mL tRNA mix (Roche, cat. no. 10109550001), 2 mM ATP (Sigma), 2 mM GTP (Sigma), 1 mM CTP (Sigma), 1 mM UTP (Sigma) and 20 mM creatine phosphate (Roche) in buffer solution.[1]

The mCherry gene was cloned into the vector pET21a. The eGFP gene was cloned into the vector pUC57.

Functionalization of PA hydrogel particles

First, acrylic acid N-hydroxysuccinimide ester was copolymerized with acrylamide to present active ester group on the polymer backbone of the hydrogel.[2] Then the gel particles were immersed in 30 mM AB-NTA solution at 65 ^{o}C overnight to incorporate AB-NTA onto polymer backbone of the PA hydrogel through the bonding with the NHS groups. The resulting hydrogel particles were transferred to 30 mM $NiCl_2$ solution for 2 hours to form the Ni^{2+}-NTA complex. Finally, the hydrogel particles were washed with water for three times and heated in air at 100 ^{o}C to be dehydrated.

The preparation of the cell-mimic particles

First, the plasmid template, ribosome, and PURE system protein factors were premixed. Then the mixture was added to the predried functionalized PA hydrogel particles and incubated on ice for 5 minutes to rehydrate the predried hydrogel particles and immobilize the PURE system factors onto the polymer backbone of the hydrogel. Finally, the feeding buffer was added to the cell-mimic particles, which were then incubated under 37 ^{o}C for protein expression. Confocal microscopy was used to observe the red fluorescence of mCherry to evaluate the protein expression level.

Continuous protein expression of the cell-mimic particles

A PDMS microfluidic device (Figure 3a) was fabricated to support the continuous protein expression. The bottom layer of the PDMS device contained a microchamber with the width of 500 μm and the height of 50 μm. The predried PA hydrogel particles were loaded in the microchamber. The top PDMS layer contained microchannels for reagent delivery. The top PDMS layer and bottom PDMS layer were treated with oxygen plasma (Plasma Prep III, SPI) and bound to form the microfluidic device.

To produce the cell-mimic particles, the PDMS device was degassed in vacuum for 10 min, then the proteinaceous factors and ribosome of the PURE system were loaded into the microchamber of the device, which contained the predried functionalized PA hydrogel particles.[3,4] The whole device was then incubated for 20 min on ice. Finally, the solution in the device was removed by vacuum, leaving the as-formed cell-mimic particles in the microchamber.

The device was put in an incubator at 37 °C and the feeding buffer was injected into the device continuously for protein expression. The feeding buffer diffused into the microchamber and fed the cell-mimic particles continuously, while the reaction products diffused out of the cell-mimic particles at the same time.

Measurement of the protein production yield

Ten predried dehydrated PA hydrogel particles were immersed in the solution containing the plasmid, ribosome, transcription and translation factors for 5 min to get rehydrated. The resulting cell-mimic particles were freeze dried. Subsequently 5 μL feeding buffer was added into the cell-mimic particles and incubated under 37 °C. The concentration of the expression product eGFP was determined by measuring the fluorescence intensity. eGFP was expressed in 5 μL buffer solution containing the PURE system as a control experiment.

IPTG regulation experiment

The non-His-tagged mCherry gene was cloned into vector pET21a, which contained the LacI gene. The T7 RNA polymerase in the PURE system transcribed the downstream of the T7 promoter to express mCherry specifically. To start the transcription and translation of LacI, 2 U *E. coli* RNA polymerase was added to the PURE system. The cell-mimic particles were loaded into a microfluidic device (Figure 3a) to perform the regulated expression of non-His-tagged mCherry. Feeding buffers containing IPTG solutions of different concentrations of 0 mM, 1 mM, 5 mM, 10 mM, and 15 mM were prepared. To test the response of the cell-mimic particles at each IPTG concentration, we first stopped the flow in the microchannel and aspirated the solution from the microchamber and the microchannel. Then we injected the feeding buffer containing the desired IPTG concentration into the microchannel and the microchamber to initiate the mCherry expression in the cell-mimic particles.

Gene oscillation experiment

0.66 μg plasmid template coding for eGFP and 0.33 μg plasmid template coding for LacI were added into 1.5 μL solution containing the proteinaceous factors and ribosome of the PURE system. Then the mixture was loaded into the microfluidic device, which contained the predried PA hydrogel particles in the microchamber for the cell-mimic particles formation. 2 mM IPTG was added into the feeding buffer for the oscillation experiment. Confocal microscopy was used to measure the eGFP fluorescence from the cell-mimic particles per 10 s.

Results

Measurement of the amount of NHS function groups in the functionalized PA gel

The measurement was based on the determination of hydroxamate.[2,5] 1 g of the predried functionalized PA gel was treated with 1 ml 0.5 M NaOH for 1 hour at 65 °C. Then the mixture was neutralized by 1 ml 0.5 M HCl, followed by the addition of 1 ml 0.85 M HCl and 0.5 ml 5 % $FeCl_3$ in 0.1 M HCl. The absorbance of the solution at 500 nm was measured. The concentration of the NHS group in PA hydrogel was found to be 27.9 mM.

Measurement of the amount of Ni^{2+}-NTA complex in the functionalized PA gel

10 µL dried functionalized PA gel was immersed into 50 µL 2.7 mg/ml His-tagged eGFP solution. After overnight incubation, 150 µL DI water was added into the mixture followed by shaking for 2 hours. Then the remaining eGFP concentration was measured in the solution, which was 0.31 mg/ml. The concentration of the His-tagged eGFP in the functionalized PA gel was calculated to be 6.5 mg/ml.

Measurement of PURE system loading efficiency in the functionalized PA gel

2.5 µL functionalized PA gel was heated in 65°C to be dehydrated. The dried PA gel was placed into a mixture of 2.5 µL DI water and 2.5 µL proteinaceous factors and ribosome solution from the PURE system, followed by incubation on ice for 5 minutes. Then, 10 µL water was added into the tube for dilution. BCA protein assay kit (Bio-Rad) was used to measure the protein concentration in the solution before loading (2.71 mg/ml) and after loading (0.67 mg/ml). As a result, the loading efficiency was 75.5% in mass.

SDS-PAGE analysis of non-His-tagged mCherry expression in the cell-mimic particles

The SDS-PAGE (Figure 2c) was performed by using a 12% PA gel. 5 µL PURE system solution without the non-His-tagged mCherry DNA template (for Lane 1), 5 µL PURE system solution with the non-His-tagged mCherry DNA template (for Lane 2), 5 µL feeding buffer containing 10 functionalized PA hydrogel particles without the PURE system (for Lane 3), and 5 µL feeding buffer containing 10 cell-mimic particles (for Lane 4) were incubated at 37 °C for 2 hours. After the preparation of the sample solutions, 2 µL solution from each sample was loaded into the SDS-PAGE setup. The detection was by Coomassie Blue staining.

Plasmid concentration change in different functionalized hydrogels

The plasmid was labelled by Evagreen and added to the gel particles, then confocal microscope was used to observe the fluorescence signal in the three different types of PA gel. As shown in Figure S1, the functionalized PA hydrogel presenting Ni^{2+}-NTA complex maintained a much higher plasmid concentration inside the gel compared to the other two gels. The plasmid trapped in the hydrogel scaffold would remain inside the gel during the continuous protein expression process.

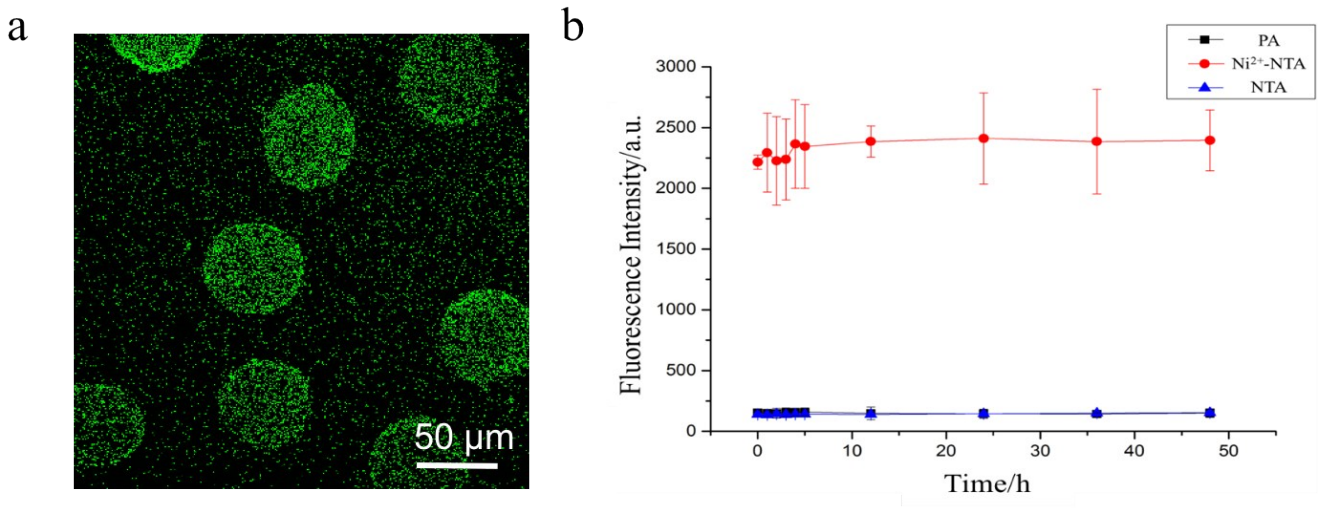

Figure S1. (a) Confocal microscopy image of the functionalized PA hydrogel containing the Evagreen labelled plasmid; (b) The plasmid concentration change inside the unfunctionalized PA hydrogel (PA), the functionalized PA hydrogel presenting NTA groups (NTA) and the functionalized PA hydrogel presenting Ni^{2+}-NTA complex (Ni^{2+}-NTA).

Diffusion of the non-His-tagged mCherry in the cell-mimic particles

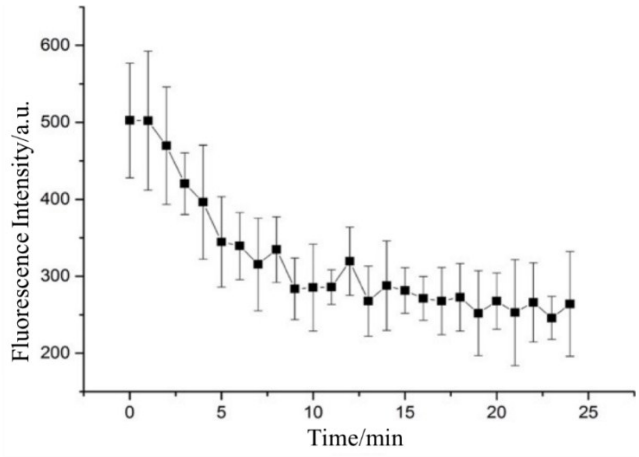

Figure S2. The decrease of the fluorescence signal from the cell-mimic particles due to the diffusion of the non-His-tagged mCherry from the particles to the surroundings. The cell-mimic particles were placed in a microchamber (Figure 3) with the continuous flow of the feeding buffer. At time zero the cell-mimic particles stopped expressing mCherry because of the temperature drop from 37°C to room temperature.

Generation of the functionalized PA gel particles

The polydimethylsiloxane (PDMS) microfluidic device with the T-junction configuration was fabricated by soft lithography to prepare the PA hydrogel particles.[6] The height of the channel of the microfluidic device was 30 µm and the width was 50 µm. 68 µmol acrylic acid N-hydroxysuccinimide ester was added to a mixture containing 8 % acrylamide, 0.8 % N,N'-methylene

bisacrylamide (MBAA), 20 % 2-hydroxy-2-methylpropiophenone, 40 % poly(ethylene glycol) 600 in 1 mL DI water. The aqueous mixture was injected into silicone oil flow in the microfluidic device to produce the aqueous droplets. The flow rate of the aqueous phase was 0.16 μL/min, while the flow rate of the oil phase was 0.32 μL/min. The droplets were exposed to UV illumination (35 mW/cm^2) for 20 seconds to initiate the photopolymerization. The resulting polymerized PA hydrogel particles were washed with water for three times to remove the unreacted reagents. The functionalized PA gel particles were stored in DMF solution at 4 ^{o}C. In the PA hydrogel, the final concentration was 8 % for acrylamide and 0.8 % for the crosslinker MBAA. In the precursor, T% (the weight percentage of acrylamide and MBAA combined) was 8.8 %, %C (the weight percentage of MBAA) was 9 %. The average pore size of the PA hydrogel was calculated to be about 12 nm.[7]

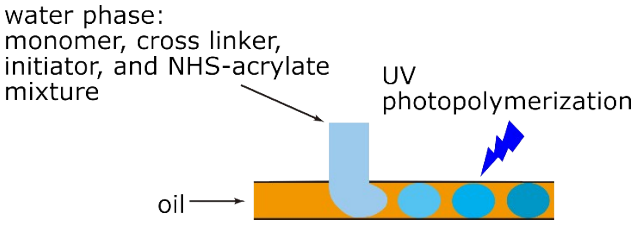

Figure S3. The schematic of the microfluidic device used for the generation of the functionalized PA gel particles.

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