Supplementary Information

Genetically engineering of *Escherichia coli* and immobilization on electrospun fibers for drug delivery purposes

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S1. Materials. All the electrophoresis agents, ampicillin, cyanuric chloride and mannose were obtained from Sigma (St. Louis, MO, USA). Plasmid pET28a(+)-pelB-EGFP was received from FulenGen Co. (Guangzhou, China), and pGEX-4T-1 vector expressing glutathione S-transferase (GST) was from GE Healthcare Bio-Sciences Corp. (Piscataway, NJ). NEB3.1 Buffer, *BamH*I and *XhoI* enzymes were received from New England Biolabs Inc. (Ipswich, MA). The premiers, buffer, Dpn I and other polymerase chain reaction (PCR) agents were procured from Takara Bio Inc. (Kusatsu, Japan). Sodium bis(2-ethylhexyl) sulfosuccinate (AOT, 96%) was purchased from Aladdin Industrial Co., Ltd. (Shanghai, China). All other chemicals were analytical grade and received from Changzheng Regents Company (Chengdu, China), unless otherwise indicated.

S2. Amplification of plasmid pelB-EGFP. The plasmid pelB-EGFP was propagated by PCR using pET28a(+)-pelB-EGFP as the template. Briefly, the sequences of pelB-EGFP premiers were designed and pelB-EGFP-BamHI-F: CGGGATCCATGAAATACCTATTGC pelB-EGFP-XhoI-R: as CCGCTCGAGTTACTTGTACAGCTC. The plasmid pET28a(+)-pelB-EGFP was incubated in a PCR buffer containing pelB-EGFP-BamHI-F (10 µM), pelB-EGFP-XhoI-R (10 µM) and dNTP mixture (2.5 µM). The amplification of DNA was performed using a 7500 Fast Real-Time PCR system (Applied Biosystems Asia, Singapore) under the optimal conditions for 30 cycles after an initial denaturation (98 °C, 4 min). Denaturation condition was set at 98 °C for 15 s, annealing at 58 °C for 20 s, and extension at 72 °C for 1 min. Final extension was performed at 72 °C for 5 min to ensure a complete synthesis of all DNA fragments. The PCR products were subjected to 1% agarose gel electrophoresis (Power PacTM Universal, Bio-Rad, Hercules, CA), and the plasmids were collected by a HiPure Gel Pure DNA Micro Kit (Magen Biotech Inc., Guangzhou, China) according to the manufacture's protocol. Fig. S1a shows the results of agarose gel electrophoresis.



Fig. S1. (a) Gel electrophoresis of plasmid pelB-EGFP and (b) segments of plasmids pGEX-4T-1 (lane 1) and pGEXM (lane 2) used for PCR identification.

S3. Construction of plasmid pGEXM. The construction of pGEXM was performed by PCR to remove GST moieties from pGEX-4T-1. The sequences of pGEX-4T-1-F and pGEX-4T-1-R were set as AACAGTATTCGGATCCCCGGAATTCCCG and CCGGGGATCCGAATACTGTTTCCTGTGT, respectively. Briefly, plasmid pGEX-4T-1 was incubated in a PCR buffer containing pGEX-4T-1-F (10 μ M), pGEX-4T-1-R (10 μ M) and dNTP mixture (2.5 μ M). After an initial denaturation at 98 °C for 3 min, the amplification of DNA was performed under the optimal conditions for 30 cycles. Denaturation condition was set at 98 °C for 15 s, annealing at 58°C for 15 s and extension at 72 °C for 5 min. Final extension was performed at 72 °C for 10 min. Then 0.4 μ L of Dpn I was added into the PCR product, followed by incubation at 37 °C for 1 h to digest the template.

The obtained plasmid was propagated on TOP10 competent cells (TransGen Biotech Inc., Beijing, China), followed by incubation in Lysogeny broth (LB) media at 37 °C for 1 h. An aliquot of the bacterial suspensions were plated on ampicillin-containing agar gel, which was incubated at 37 °C overnight. Then 1 or 2 clones were transferred into LB media containing 0.1g/L ampicillin overnight, and the plasmids were retrieved by a HiPure Plasmid mini Kit (Magen Biotech Inc., Guangzhou, China) according to the manufacture's protocol.

The obtained plasmids were characterized by PCR using pGEXM as the template. The sequences of pGEXM-F and pGEXM-R were designed as GCTCAAGGCGCACTCCCGTTCTG and CCGGGAGCTGCATGTGTCAGAGG, respectively. The plasmid pGEXM was incubated in PCR buffer containing pGEXM-F (10 μ M), pGEXM-R (10 μ M) and dNTP mixture (2.5 μ M). The amplification of DNA was performed under the optimal conditions for 30 cycles, including denaturation at 98 °C for 15 s, annealing at 58 °C for 15 s, and extension at 72 °C for 90 s. Final extension was performed at 72 °C for 5 min. The PCR products were subjected to 1% agarose gel electrophoresis. Fig. S1b shows the results of agarose gel electrophoresis. The plasmid sequence was analyzed by Taihe Biotech. Co., Ltd. (Beijing, China) and listed as follows.

AATTAGCTTATCGACTGCACGGTGCACCAATGCTTCTGGCGTCAGGCAGCCATCGGAAGC TGTGGTATGGCTGTGCAGGTCGTAAATCACTGCATAATTCGTGTC<u>GCTCAAGGCGCACTCC</u> <u>CGTTCTG</u>GATAATGTTTTTTGCGCCGACATCATAACGGTTCTGGCAAATATTCTGAAATGA GCTGTTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAATTTC ACACAGGAAACAGTATTCGGATCCCCGGAATTCCCGGGTCGACTCGAGCGGGCCGCATCGT GACTGACTGACGATCTGCCTCGCGCGTTTCGGTGATGACGGTGAAA<u>ACCTCTGACACATG</u> <u>CAGCTCCCGGA</u>GACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGT CAGGGCGCGTCAGCGGGTGTTGGCGGGGTGTCGGGGCGCAGCCATGACCAGTCACGTAGC GATAGCGGAGTGTATAATTCTTGAAGACGAAAGGGCCTCGTGATACGCCTATTTTTATAG G

The underlined sequences among the pair of specific primers showed that the GST barcode was kicked off successfully. Site-directed mutagenesis was introduced to delete the unwanted 672 bp sequences from initiation codon (ATG) to *BamH*I restriction enzyme cutting site (GGATCC), belonging to the GST barcode. The open reading frame (ORF) of the transformed vector should start from the exogenous code fragment ATG behind the *BamH*I restriction enzyme cutting site.

S4. The Sequence of plasmid pGEXM-pelB-EGFP. The sequence of plasmid pGEXM-pelB-EGFP was listed as follows. The unlined 66 bases were from pelB leader, followed by the code area of EFGP sequence till TAA termination codon.

TGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGTATTCGGATCC<u>ATGAAA</u> <u>TACCTATTGCCTACGGCAGCCGCTGGATTGTTATTACTCGCTGCCCAACCAGCGATGGCT</u> TGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACG GCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTAC GGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACC CTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAG CAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCT TCAAGGACGACGGCAACTACAAGACCCGCGCGCGAGGTGAAGTTCGAGGGCGACACCCTG GTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCA CAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGA ACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCG CCGACCACTACCAGCAGAACACCCCCATCGGCGACGACGGCAGCGTGCAGCTCG ACTACCTGAGCACCCAGTCCGACCACCAACGTCCTAGGAGGACGGCGATCACATGG TCCTGCTGGAGTTCGTGACCGCCCGCGGGATCACTCTCGGCATGAAGCGCGATCACATGG ACAGCTGGAGTTCGTGACCGCCGCGCGGGATCACTCTCGGCATGGACGACGACGACGACGAGCAGCAGCACATCCAACG ACTACCTGAGCACCCCAGTCCGCCCGGGATCACTCTCGGCAAGGACGAGCGGACGACCACATGG TCCTGCTGGAGTTCGTGACCGCCGCGCGGGATCACTCTCGGCATGGACGACGACGAGCAGCAGCAGCAGAGA AA

S5. Preparation and characterization of NaCl microparticles. NaCl microparticles were prepared using reverse microemulsions as described previously with some modifications.¹ Briefly, 0.444 g AOT was dissolved in 10 mL n-heptane, and 2.2 mg CaCl₂ was dissolved in 0.2 mL formamide solution. The CaCl₂ solution was added dropwise to the AOT solution, followed by stirring for 1 h at room temperature. Acetone was added to break the emulsion for 1 h, followed by ultrasonication for 5 min to disperse the formed microparticles. The suspensions were centrifuged to collect the microparticles, followed by acetone washing twice and vacuum dried. The average size and size distribution of microparticle were detected by dynamic light scattering (DLS, Nano-ZS90, Malvern Ltd., U.K.). Through changing the amount of AOT used, NaCl microparticles with average sizes of around 200 (PDI: 0.21) and 600 nm (PDI: 0.28) were prepared. **Fig. S2** shows the DLS images of NaCl microparticles.



Fig. S2. (a) DLS analysis of NaCl microparticles with average sizes of around 200 and (b) 600 nm.

S6. Synthesis of cyanuric chloride-mannose conjugates. Cyanuric chloride-mannose conjugates were prepared through reaction of hydroxyl groups from mannose and cyanogen chloride from cyanuric chloride as described previously.² Briefly, mannose (240 mg, 1.33 mmol) and NaOH (79.2 mg, 1.98 mmol) were dissolved in water (10 mL), followed by the addition of cyanuric chloride (240 mg, 1.30 mmol) solution in acetone (30 mL). After stirring for 8 h at 4 °C, the mixed solution was distilled in the rotary evaporator to remove acetone. The crude product was chromatographed on silica gel with petroleum ether (60–90 °C)/ethyl acetate (5:2, v/v) to remove the unreacted reactants, followed by freeze drying to collect a white powder. **Fig. S3** shows the Fourier transform infrared (FTIR) spectra of cyanuric chloride-mannose conjugates recorded on a Nicolet 5700 spectrometer (Thermo Electron, Madison, WI)



Fig. S3. FITR spectra of cyanuric chloride (CC), mannose and CC-mannose conjugates.

S7. Degrdation behaviors of PELA fibers. The degradation behavior of prstine PELA fibers and porous fibers after removal of NaCl microparticles were determined as described previously with some modification.³ Briefly, around 60 mg of PELA or porous PEL fibers were incubated at 37 °C in 40 mL phosphate buffered saline (PBS; pH 7.4) or LB media containing 10⁶ cfu of *E. coli* strain Nissle 1917 (EcN). In the process of degradation, EcN media were refreshed to keep EcN activity. At specified intervals, fibers were retrieved and rinsed with distilled water to remove residual buffer salts, and vacuum dried. The molecular weight of residual fibers was detected by gel permeation chromatography (GPC; Waters 2695 and 2414, Milford, MA) using polystyrene as standard. As shown in **Fig. S4**, After incubation in PBS for 2 weeks, the molecular weight residuals were about 94.0% and 90.1%, respectively, and no signifiant difference was observed between them (p > 0.05). The slightly higher degradation rate was due to the higher water diffusion rate into porous fibers. In addition, the molecular weight remaining of PELA and porous PELA fibers was around 92.4% and 89.9% after incubation in EcN-containing media for 2 weeks, respectively. There was no significant difference in the molecular weight loss after degradation in PBS and EcN-containing media for both PELA and porous PELA fibers (p > 0.05).



Fig. S4. The molecular weight retention of prstine PELA fibers and porous PELA fibers after removal of NaCl microparticles after incubation in pH 7.4 PBS or bacteria-containing media at 37 $^{\circ}$ C (n = 3).

REFERENCES

- L. Zhang, H. M. Cheng, J. M. Ma. Preparation of NaCl nanoparticles in nonaqueous reverse microemulsion. *Acta Phys.-Chim. Sin.* 2002, 18, 79–81.
- (2) K. Liang, Y. Chen. Elegant chemistry to directly anchor intact saccharides on solid surfaces used for the fabrication of bioactivity conserved saccharide microarrays. *Bioconjugate Chem.* 2012, 23, 1300–1308.
- (3) W. Cui, X. Li, S. Zhou, J. Weng, Degradation patterns and surface wettability of electrospun fibrous mats. *Polym. Degrad. Stabil.* 2008, 93, 731–738.