Electronic Supplementary Information

Microfluidic spinning induced heterotypic bead-on-string fibers for dual-cargo release and wound healing

Qiu Huang,[a] Fukun He,[a] Jiafei Yu,[b] Jing Zhang,[a] Xiangyun Du,[a] Qing Li,[a] Gefei Wang,[b] Ziyi Yu[a] and Su Chen*[a]*

[a] State Key Laboratory of Materials-Oriented Chemical Engineering, College of Chemical Engineering, Jiangsu Key Laboratory of Fine Chemicals and Functional Polymer Materials, Nanjing Tech University, 30 Puzhu South Road, Nanjing 211816, P. R. China. E-mail: ziyi.yu@njtech.edu.cn; chensu@njtech.edu.cn; Fax: +86-25-83172258; Tel: +86-25-83172258;
[b] Department of General Surgery, Jinling Hospital, Nanjing Medical University, Nanjing 210002, P. R. China.

Experimental Section

Preparation of PBS (pH=7.4) solution

7.16 g $Na_2HPO_4 \cdot 12H_2O_{(S)}$ (0.02 mol) was dissolved in a small quantitative of deionized water, and then dilute to a 100 mL volumetric flask to make a 0.2 mol/L disodium hydrogen phosphate solution. 2.72 g $KH_2PO_{4\ (S)}$ (0.02 mol) was dissolved, and then dilute to a 100 mL volumetric flask to prepare a 0.2 mol/L potassium dihydrogen phosphate solution. Mixing the 81 mL 0.2 mol/L disodium hydrogen phosphate solution with the 19 mL 0.2 mol/L potassium dihydrogen phosphate solution, the 0.8 g NaCl and 0.02 g KCl were added to the mixed solution, and then the solution of PBS (pH=7.4) was obtained.

Drawing of the standard curve of bovine serum albumin (BSA).

The 100 mg BSA was accurately weighed by an analytical balance, and then the model drug was dissolved in a suitable quantitative of PBS solution, then it was diluted to a 100 mL volumetric flask to prepare the 1 mg/mL BSA solution. Usually, to prepare 0 mg/mL (pure PBS solution), 0.2 mg/mL, 0.4 mg/mL, 0.6 mg/mL, 0.8 mg/mL, 1.0 mg/mL BSA solution, 6 clean tubes were needed, and they were numbered 0-5. The absorbance of BSA solution at different concentrations was

measured at 280 nm with a dual beam UV-VIS spectrophotometer.

Drawing of the standard curve of ibuprofen.

The 50 mg ibuprofen was accurately weighed by an analytical balance, and then the model drug was dissolved in a suitable quantitative of PBS solution, then it was diluted to a 100 mL volumetric flask to prepare the 0.5 mg/mL ibuprofen solution. Usually, to prepare 0 mg/mL (pure PBS solution), 0.1 mg/mL, 0.2 mg/mL, 0.3 mg/mL, 0.4 mg/mL, 0.5 mg/mL ibuprofen solution, 6 clean tubes were needed, and they were numbered 0-5. The absorbance of ibuprofen solution at different concentrations was measured at 265 nm with a dual beam UV-VIS spectrophotometer.

Supplementary Figures

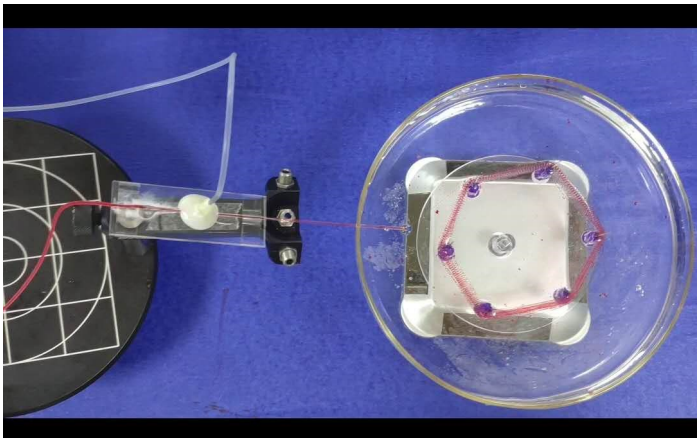

Figure S1 Continuous preparation of heterotypic bead-on-string microfibers by means of microfluidic spinning and automated collection methods

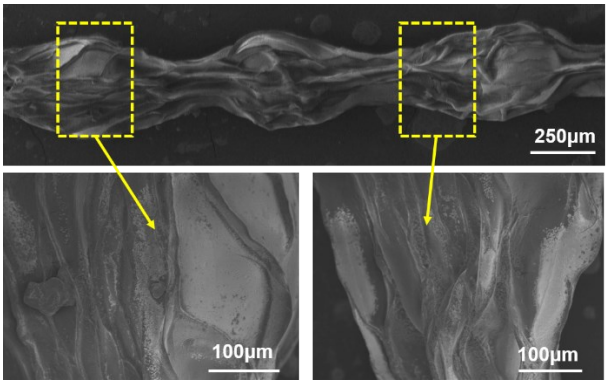

Figure S2 SEM of dual-cargo-loaded heterotypic bead-on-string microfibers after freeze-drying

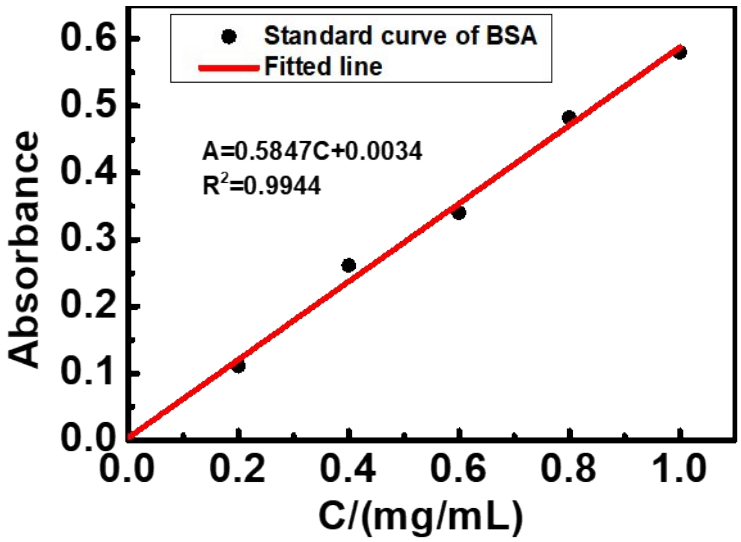

Figure S3 Standard curve of bovine serum albumin (BSA)

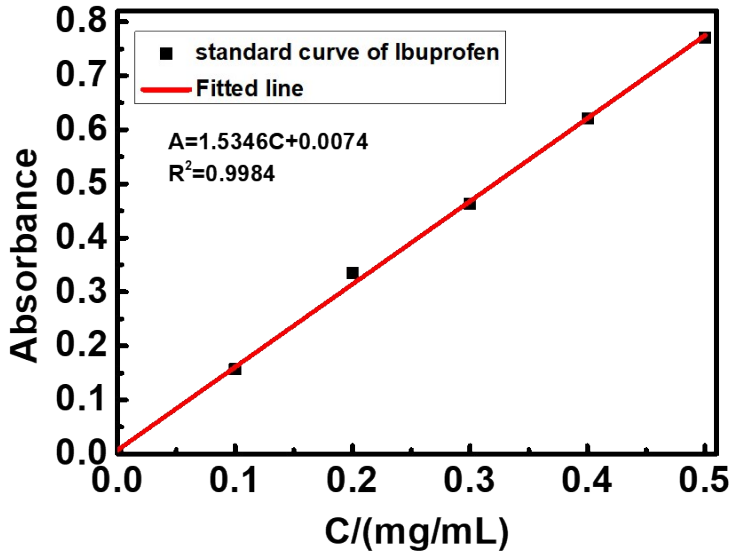

Figure S4 Standard curve of ibuprofen

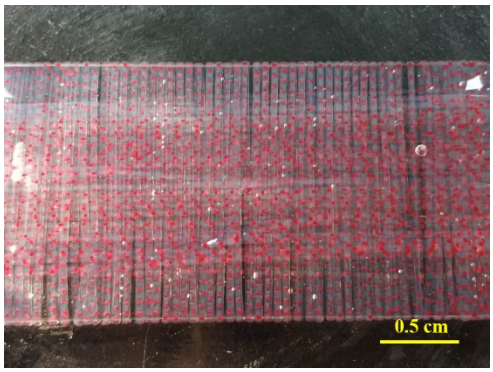

Figure S5 Ordered weaving of dual-cargo-loaded heterotypic bead-on-string microfibers

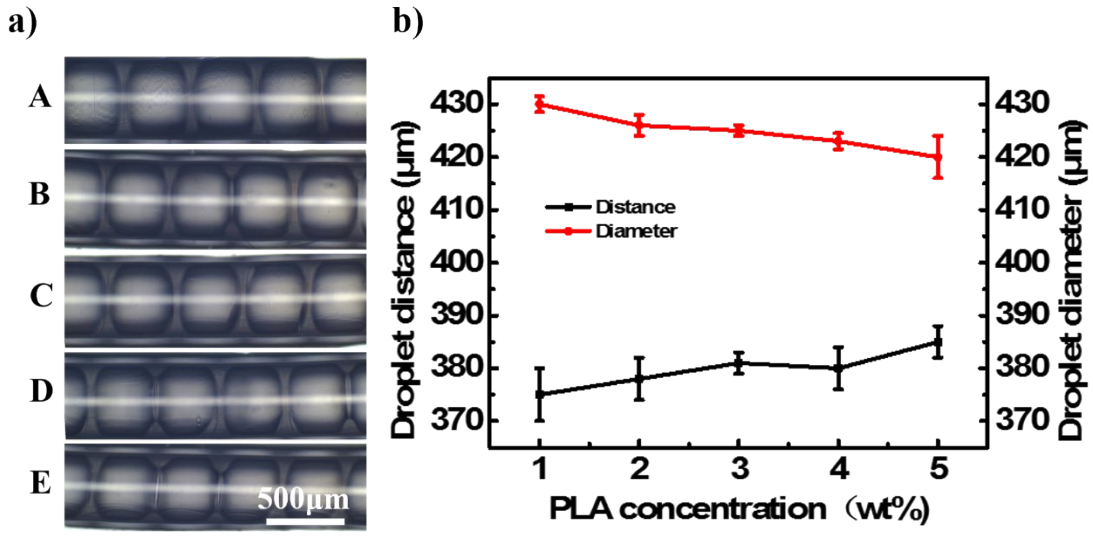

Figure S6 Morphology change of bead-on-string microfibers as a function of the PLA concentration. a) Optical micrographs of bead-on-string microfibers as a function of the PLA concentration. b) Droplet distance and diameter as a function of the PLA concentration.

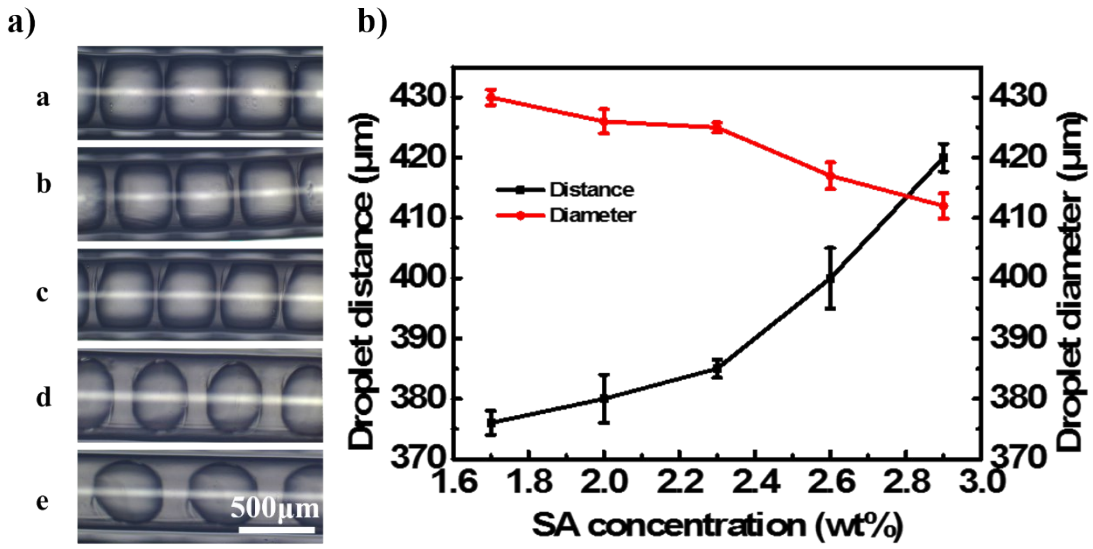

Figure S7 Morphology change of bead-on-string microfibers as a function of the SA concentration. a) Optical micrographs of bead-on-string microfibers as a function of the SA concentration. b) Droplet distance and diameter as a function of the SA concentration.

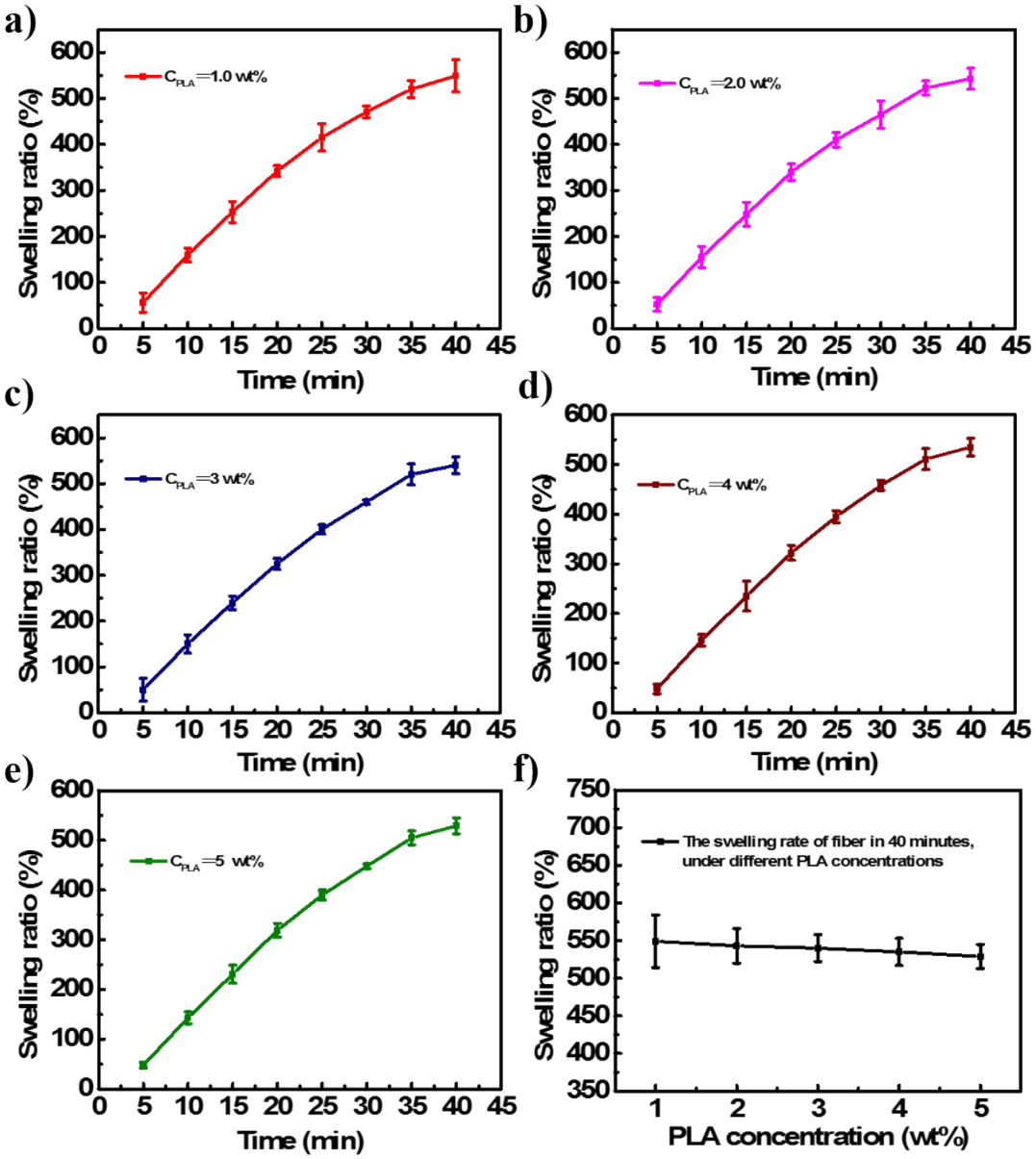

Figure S8 Swelling ratio of bead-on-string microfibers as a function of the time under different concentration of PLA. (a-e) Volume change over time for bead-on-string microfibers as a function of the PLA concentration at 1.0 wt%, 2.0 wt%, 3.0 wt%, 4.0 wt%, and 5 wt%, respectively. (f) The swelling ratio of bead-on-string microfibers as a function of PLA concentration in 40 minutes.

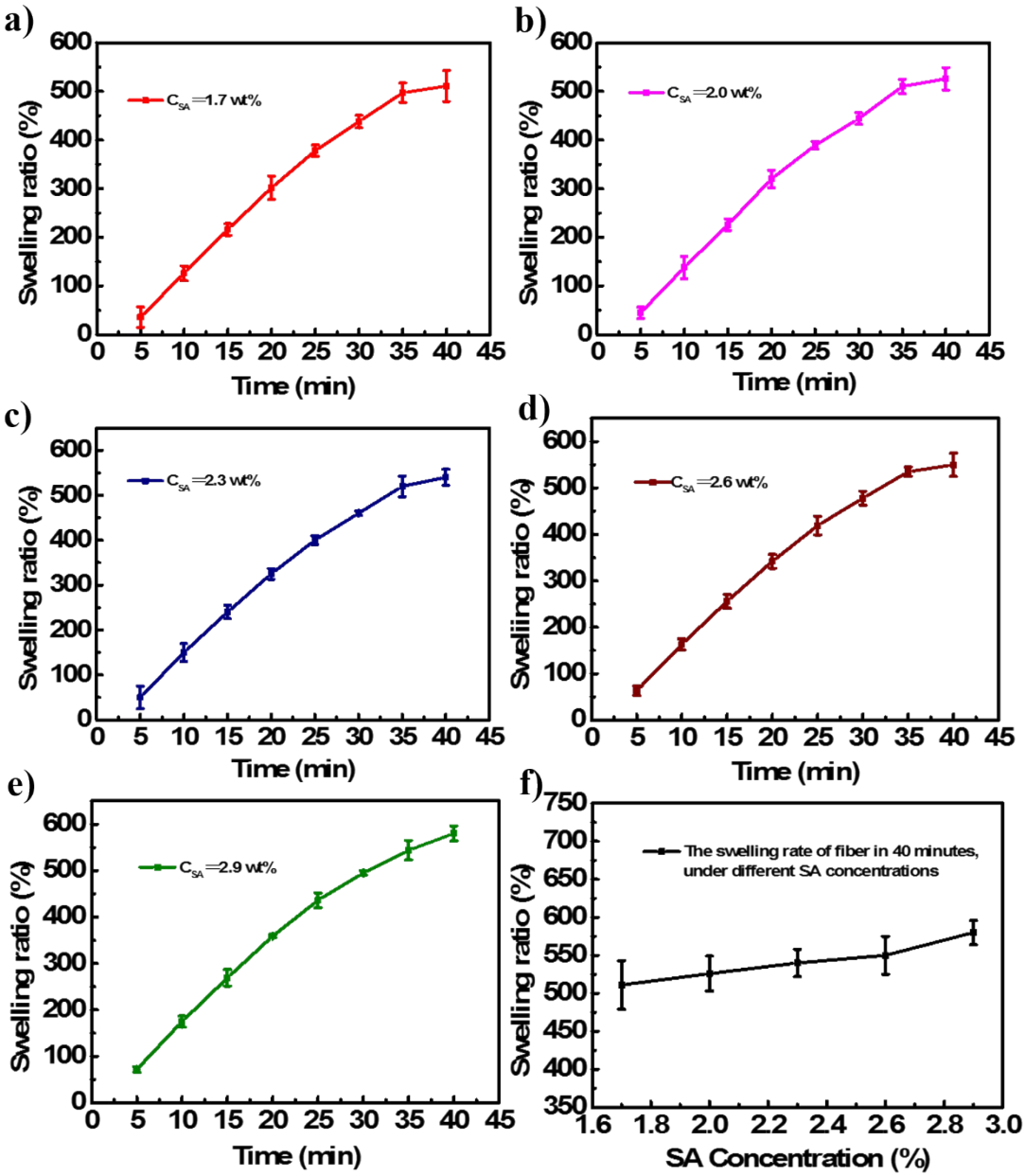

Figure S9 Swelling ratio of bead-on-string microfibers as a function of the time under different concentration of SA. (a-e) Volume change over time for bead-on-string microfibers as a function of SA concentration at 1.7 wt%, 2.0 wt%, 2.3 wt%, 2.6 wt % and 2.9 wt%, respectively. (f) Swelling ratio of bead-on-string microfibers as a function of SA concentration in 40 minutes.

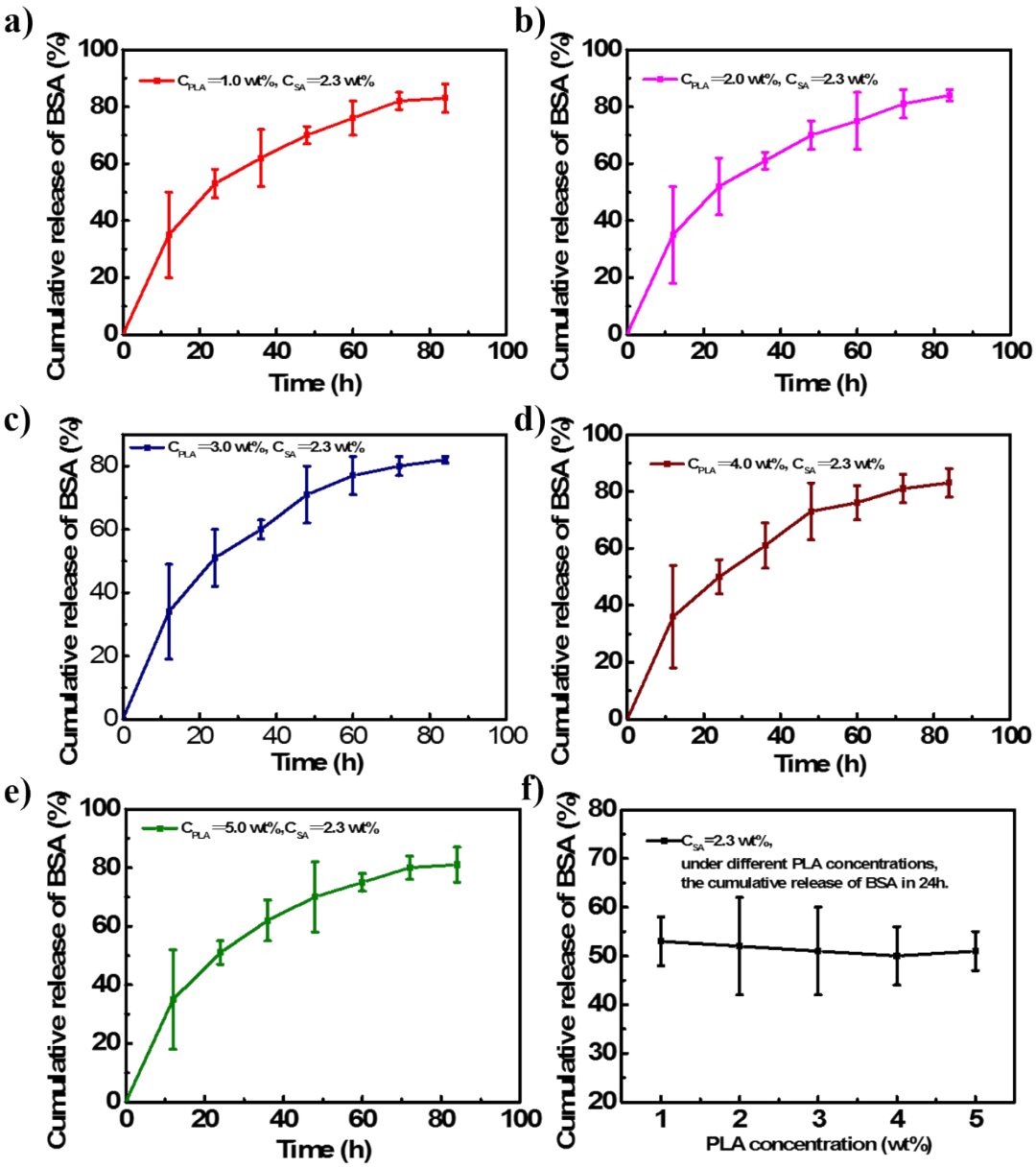

Figure S10 Cumulative release of BSA as a function of the time under different concentration of PLA. (a-e) Cumulative release of BSA over time for bead-on-string microfibers as a function of PLA concentration at 1.0 wt%, 2.0 wt%, 3.0 wt%, 4.0 wt % and 5.0 wt%, respectively. (f) The cumulative release of BSA as a function of PLA concentration in 24h.

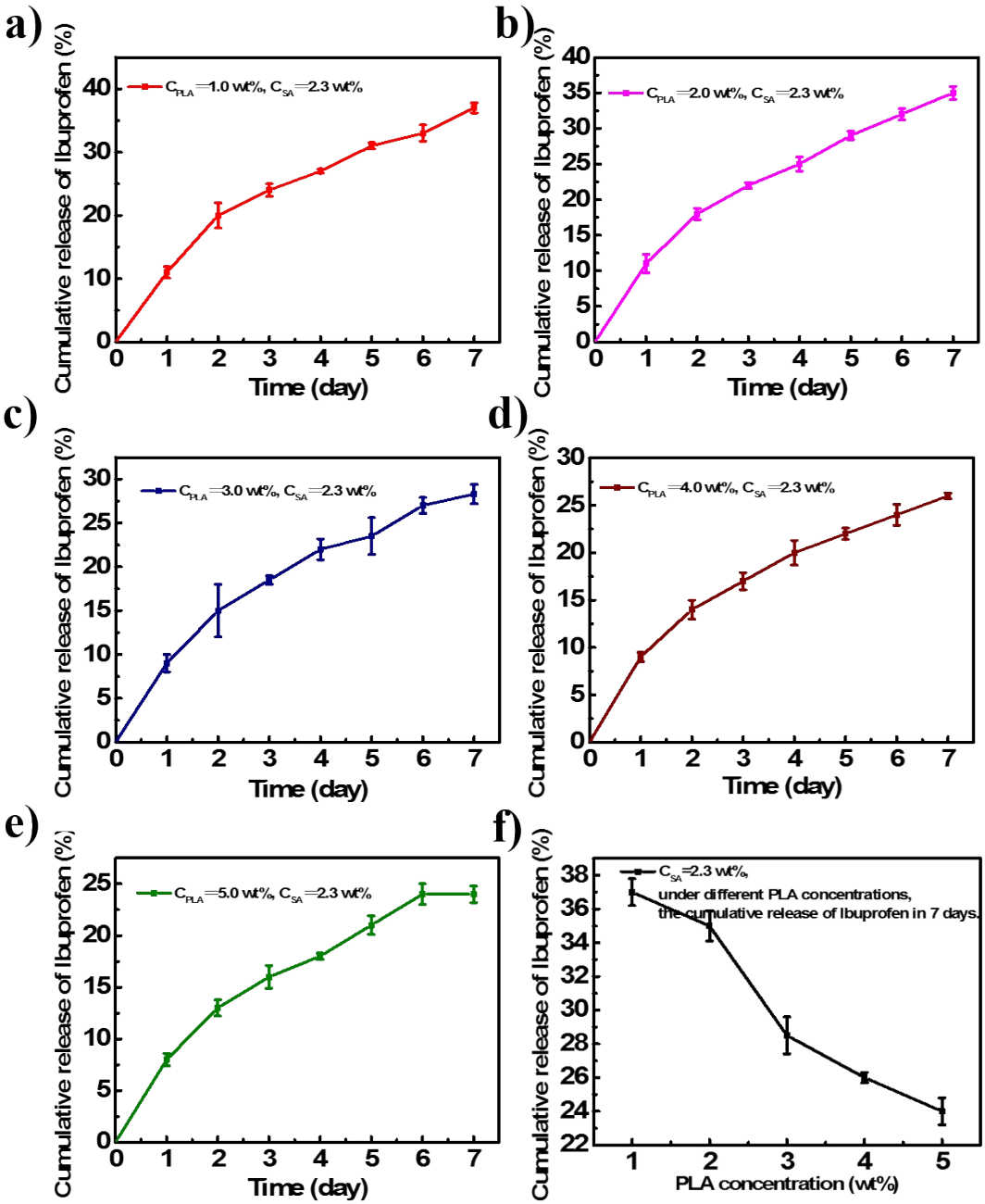

Figure S11 Cumulative release of ibuprofen as a function of the time under different concentration of PLA. (a-e) Cumulative release of ibuprofen over time for bead-on-string microfibers as a function of PLA concentration at 1.0 wt%, 2.0 wt%, 3.0 wt%, 4.0 wt % and 5.0 wt%, respectively. (f) The cumulative release of ibuprofen as a function of PLA concentration in 7 days.

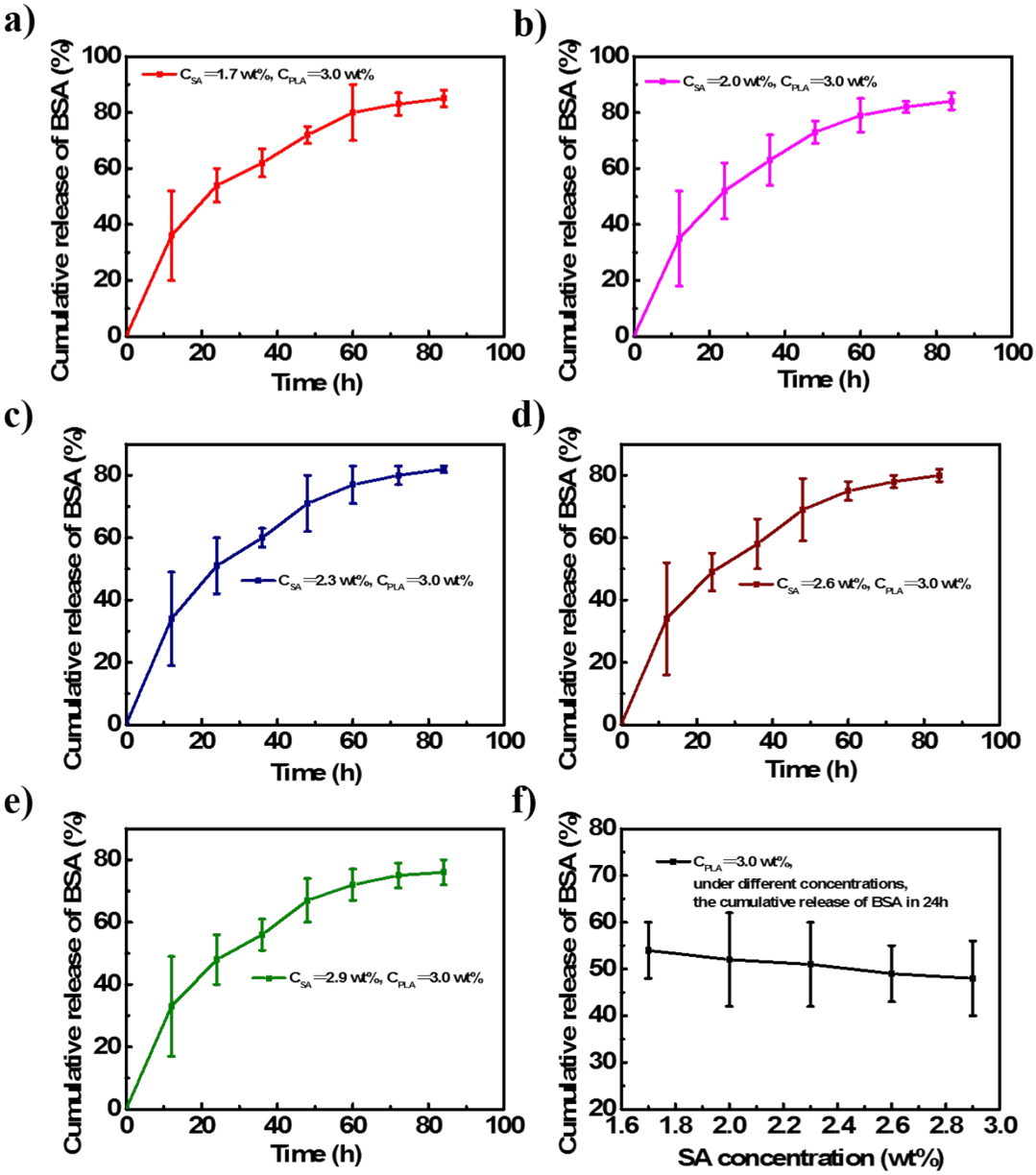

Figure S12 Cumulative release of BSA as a function of the time under different concentration of SA. (a-e) Cumulative release of BSA over time for bead-on-string microfibers as a function of SA concentration at 1.7 wt%, 2.0 wt%, 2.3 wt%, 2.6 wt % and 2.9 wt%, respectively. (f) The cumulative release of BSA as a function of SA concentration in 24h.

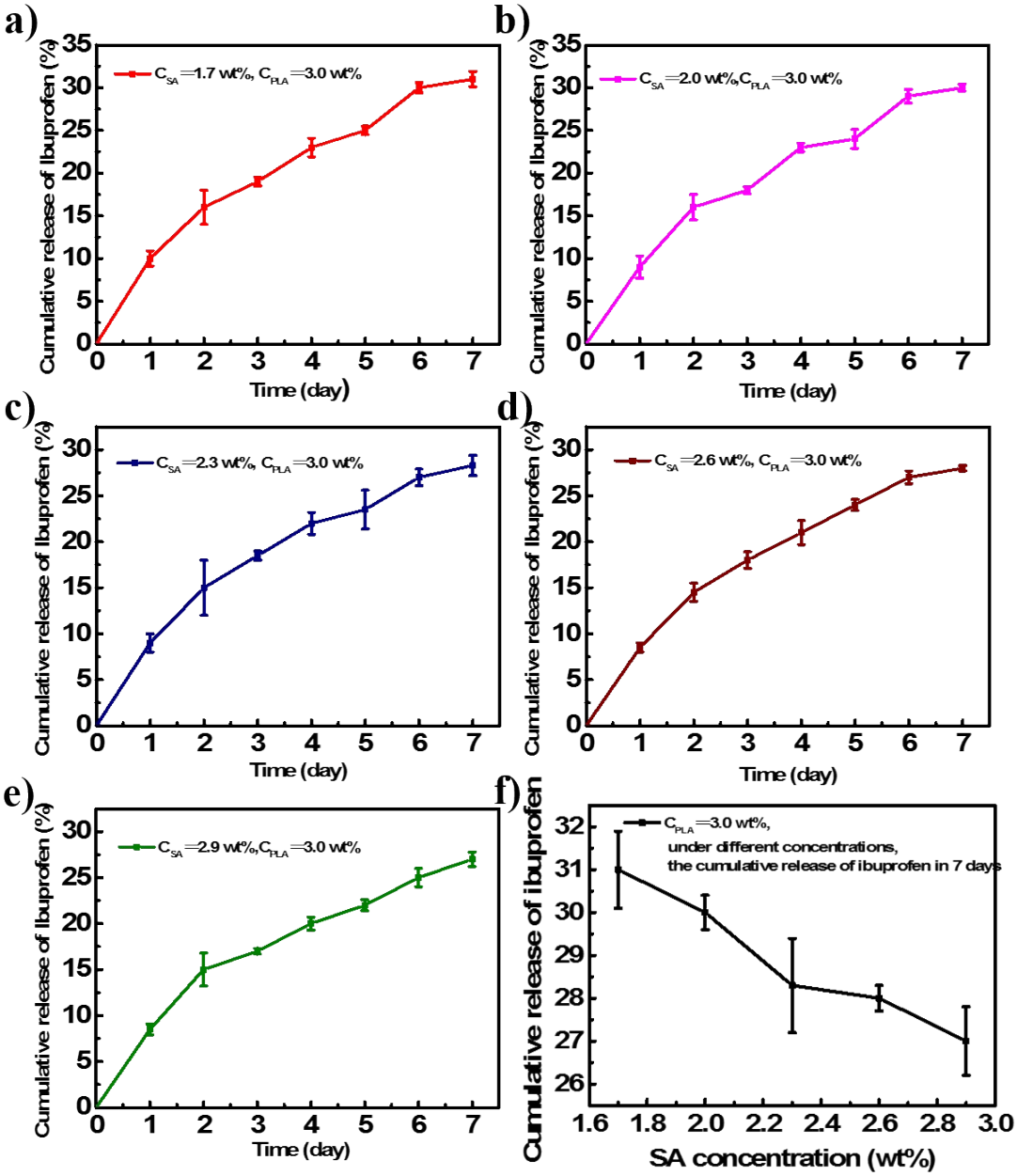

Figure S13 Cumulative release of ibuprofen as a function of the time under different concentration of SA. (a-e) Cumulative release of ibuprofen over time for bead-on-string microfibers as a function of SA concentration at 1.7 wt%, 2.0 wt%, 2.3 wt%, 2.6 wt % and 2.9 wt%, respectively. (f) The cumulative release of ibuprofen as a function of SA concentration in 7 days.

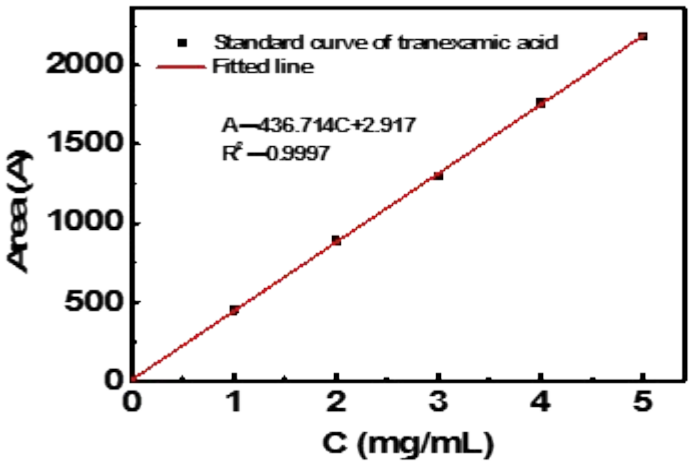

Figure S14 Standard curve of tranexamic acid.

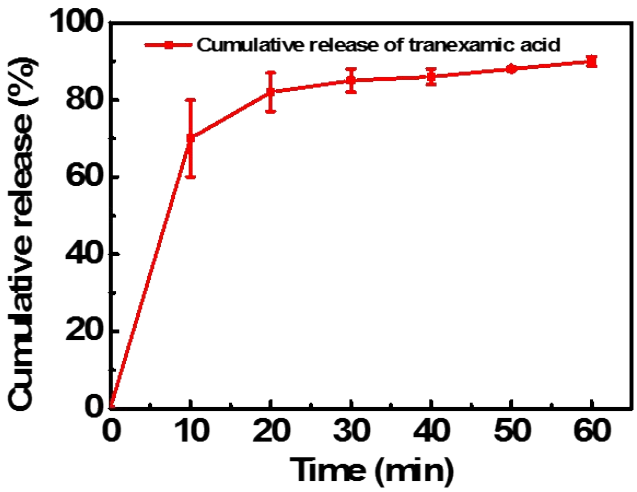

Figure S15 Release profile of tranexamic acid in bead-on-string microfibers as a function of time.

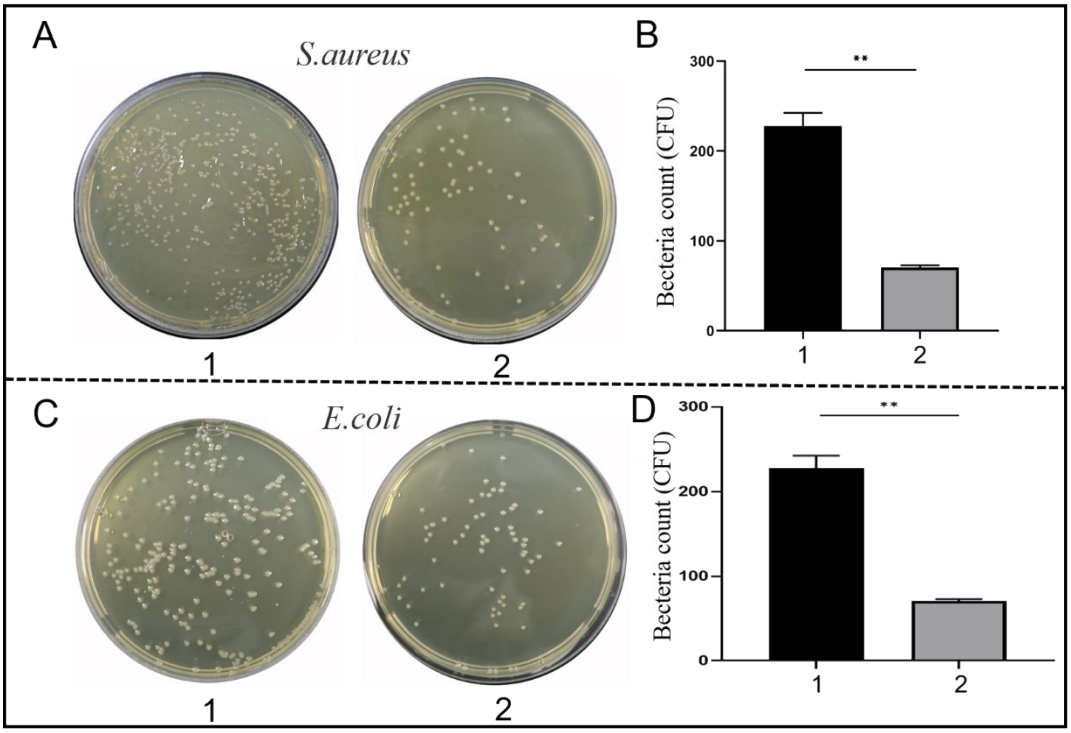

Figure S16 Antibacterial activities against strains of *Staphylococcus aureus* and *Escherichia coli*. Antibacterial activities against strains of *Staphylococcus aureus* and *Escherichia coli*. (A)1: Colony formation after treatment of PBS; 2: Colony formation after treatment of scaffold. (B) *Staphylococcus aureus* colony count. (C) 1: Colony formation after treatment of PBS; 2: Colony formation after treatment of scaffold. (D) *Escherichia coli* colony count. **, $p < 0.01$. (p-value was calculated using unpaired t test with Welch's correction.)

Table 1 Mechanical strength of the bead-on-string microfibers

Breaking strength/(cN/dtex)	breaking elongation/%
2.97	12.243

Supplementary discussions

Preparation of heterotypic bead-on-string microfibers

To study the properties of bead-on-string microfibers in detail, we further investigated the effects of carrier (alginate and PLA) concentration on the droplet diameter and swelling rate at a constant fluid flow rate. The experiment result shows that, when the sodium alginate concentration was 2.3 wt% and the flow rate was 10:6 (mL/h: mL/h), the diameter of the droplets decreased and the droplet spacing increased, with the increase of PLA concentration, as shown in Fig.S6. This may be caused by the increase in the viscous resistance of the internal phase solution. In addition, when the PLA concentration was 3.0 wt% and the flow rate was 10:6 (mL/h: mL/h), the diameter of the droplets decreased and the droplet spacing increased, with the increase of SA concentration, as shown in Fig.S7. This may be caused by the viscous resistance of the high-concentration external phase solution to the internal phase solution.

The swelling rate experiment shows that, when the sodium alginate concentration was 2.3 wt% and the flow rate was 10:6 (mL/h: mL/h), with the increase of the PLA concentration, the swelling rate of the fiber is basically unchanged, as shown in Fig. S8. This may be caused by the fact that PLA is a good hydrophobic material. However, when the PLA concentration was 3.0 wt% and the flow rate was 10:6 (mL/h: mL/h), the swelling rate increases with the increase of sodium alginate concentration, as shown in Fig. S9. This may be caused by the increase in the composition of SA (water-absorbing material) in the fiber as the concentration of sodium alginate increases.

We also investigated the effect of the concentration of the carrier (sodium alginate and PLA) on the sustained release of the dual-drugs. The experiment result shows that, when the SA concentration was 2.3 wt% and the flow rate was 10:6 (mL/h: mL/h), the release rate of BSA in SA phase was independent of PLA concentration, as shown in Fig. S10. This may be due to the absence of bond cooperation in the fiber and the PLA is encapsulated in the sodium alginate phase. Therefore, the concentration of PLA will not affect the release of BSA in the SA phase. Secondly, when the SA concentration was 2.3 wt% and the flow rate was 10:6 (mL/h: mL/h), the release rate of ibuprofen in the PLA phase decreases with the increase of PLA concentration, as shown in Fig. S11. This may be due to the binding effect of hydrophobic PLA on ibuprofen. In addition, when the PLA concentration was 3.0 wt% and the flow rate was 10:6 (mL/h: mL/h), with the increase of the SA concentration, the release rate of BSA in the SA phase is basically unchanged, as shown in Fig. S12. This may be due to the good solubility of BSA in PBS solution at pH=7.4, and the weak binding capacity of calcium alginate to BSA. Fourthly, when the PLA concentration was 3.0 wt% and the flow rate was 10:6 (mL/h: mL/h), with the increase of the SA concentration, the release rate of ibuprofen in the PLA phase is decrease, as shown in Fig. S13. This may be due to the poor solubility of ibuprofen in PBS solution at pH=7.4, and part of BSA is adsorbed in the calcium alginate phase. In other words, with the increase of sodium alginate concentration, the calcium alginate composition increases, and the adsorbability to ibuprofen becomes stronger. In addition, through quality analysis, the dosage of drugs can be accurately controlled. Among that, the load of the drug in the fiber is determined by the content of the drug in the solution.

Cargo release from heterotypic bead-on-string microfibers

We also investigated the release process of encapsulated drugs (tranexamic acid, nano-silver, emu oil) in the fiber. The fibers containing 100 mg tranexamic acid were immersed in (pH=7.4) PBS solution, and the supernatant was taken out within the same time interval (10 min), and then tested and analyzed by high-performance liquid chromatography (HPLC, LC8600). Among them, a C18 column was used as the chromatographic column. A pH=2.5, 0.23 wt% sodium laurilsulfate solution-methanol (60:40 wt%) was used as the mobile phase. The fluid flow rate and temperature were set at 1.5 mL/min and 30 °C, respectively, and the peak area of tranexamic acid was measured at the wavelength of 220 nm. Then, according to the standard curve of tranexamic acid (Fig. S14), the cumulative release amount of tranexamic acid over time was determined. As show in Fig. S15, the release rate of tranexamic acid was rapid. This may be due to the low molecular weight of tranexamic acid and strong hydrophilicity.

In addition, the cumulative release of silver nanoparticles in different time periods was measured by ultraviolet spectrophotometer. The experimental results show that the cumulative release data of silver nanoparticles remain unchanged in different time. This may be due to the diameter of silver nanoparticles (25±5 nm) wrapped in calcium alginate phase is larger than the pore

of the hydrous CaA hydrogel (<20 nm).[16] In addition, the slow degradation of calcium alginate in PBS solution also made it difficult for silver nanoparticles to be released.

Emu oil is mainly composed of fatty acids, and oleic acid accounts for about 43-46% of the fatty acid compositions. Among them, there are also linoleic acid, palmitic acid, stearic acid and linolenic acid.[17] Due to the complex composition of emu oil and its insolubility with PBS solution, we cannot accurately determine the release curve of emu oil at present.

This kind of microfiber is composed of hydrophilic and hydrophobic parts, which can realize the simultaneous loading of dual drugs. The drugs loaded on the fiber can be released in two different ways, and the drug release rate can be effectively controlled by regulating the composition of the two parts of materials. In addition, compared with bulk and microsphere morphology drug-loading materials, this kind of microfiber has the advantages of large specific surface area, good weaving performance, high transparency, good biocompatibility and so on, and the skin scaffold prepared has good air permeability and bi-directional tensile property, and we can observe wound healing without removing the skin scaffolds.

Antibacterial properties of dual-cargo loaded heterotypic bead-on-string microfibers

Staphylococcus aureus and *Escherichia coli* were added to two equal amounts of PBS, two kinds of bacterial turbid liquids were prepared base on McFarland standards, and then the two kinds of bacterial turbid liquids were diluted 100 times. To further investigate the antibacterial properties of the fiber scaffold, respectively suck 10 mL of the diluted *Staphylococcus aureus* (or *Escherichia coli*) bacterial turbid solution into two culture flasks, add an appropriate amount of dual-cargo loaded heterotypic bead-on-string microfibers to one of the culture flasks, and the other without anything as a control group, and incubate for 12 hours. Then, the bacteria in the supernatant were inoculated on a mannitol salt agar plate (Bizheng Biotech CO. LTD, China) at 37°C. After 24 hours, the number of bacterial colonies was counted.

Mechanical properties test of the heterotypic bead-on-string microfibers

The mechanical strength of calcium alginate heterotypic bead-on-string microfibers was measured by single fiber tensile strength tester (MJDS-5A). The tensile distance was 10mm, the pretension was 2cN, and the tensile speed was 20 mm/min. The mechanical properties are shown in Table1.