ADVANCED FUNCTIONAL MATERIALS

Supporting Information

for *Adv. Funct. Mater.,* DOI: 10.1002/adfm.201806883

Injectable Self-Healing Antibacterial Bioactive Polypeptide-Based Hybrid Nanosystems for Efficiently Treating Multidrug Resistant Infection, Skin-Tumor Therapy, and Enhancing Wound Healing

*Li Zhou, Yuewei Xi, Yumeng Xue, Min Wang, Yanle Liu, Yi Guo, and Bo Lei**

Supporting Information

Injectable Self-Healing Antibacterial Bioactive Polypeptide-based Hybrid Nanosystems for Efficiently Treating Multidrug Resistant Infection, Skin Tumor Therapy and Enhancing Wound Healing

Li Zhou [a, b], Yuewei Xi [b], Yumeng Xue [b], Min Wang [b], Yanle Liu [a], Yi. Guo [b, d], Bo Lei [a, b, c] *

[a]Key Laboratory of Shaanxi Province for Craniofacial Precision Medicine Research, College of *Stomatology, Xi'an Jiaotong University, Xi'an, 710049, China*

[b] Frontier Institute of Science and Technology, X*i'an Jiaotong University*, *Xi'an, 710049, China*

[c] Instrument Analysis Center, Xi'an Jiaotong University, *Xi'an, 710049, China*

[d] Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI 48109, USA

* To whom correspondence should be addressed.

Professor Bo Lei, Tel.:+86-29-83395361.

E-mail: rayboo@xjtu.edu.cn

Experimental section

1.1. Synthesis and characterizations of F127-Phe-CHO and F127-EPL

F127-sulfanilic acid ester (F127-OTs) was synthesized as reported previously[1]. Dibenzaldehy determinated F127 (F127-Phe-CHO) was prepared in a similar method as described previously[2]. Briefly, 2.5 g (0.2 mmol) F127-OTs was dissolved in 50 mL DMF, 4-hydroxybenzaldehyde (0.11 g, 0.9 mmol) and K_2CO_3 (0.12 g, 0.9 mmol) were added followed. The mixture was stirred at 80 °C for 72 h and then cooled to room temperature. The reaction solution was extracted with CH_2Cl_2 after adding 50 mL H_2O. The organic layer was dried over $MgSO_4$, concentrated and precipitated in cold diethyl ether (at ten times excess). After filtration, F127-Phe-CHO was dried at room temperature under vacuum for 24 h (Yield: 90%).

F127-EPL (FEPL) copolymer was synthesized in a similar method as described previously[1]. Briefly, to obtain FEPL copolymer, 4.2 g EPL (1.2 mmol) and F127-OTs (0.5 mmol) were dissolved in 20 mL H_2O and 60 mL DMSO, respectively. Then, the F127-OTs solution was added to the EPL solution and the reaction was carried out at 60 °C for 72 h. The mixture was cooled to room temperature and dialyzed against deionized water using 5000 MWCO membrane for 3 days. The FEPL copolymer was obtained by freeze-drying and stored at 4 ^{o}C (Yield: 80%).

1.2 Synthesis of BGN@PDA

BGN (bioactive glass nanoparticle) was synthesized as reported previously[3]. Briefly, 1 g DDA was dissolved in 6.25 mL H_2O and 20 mL ethanol mixed solution. Then, 0.5 mL TEOS was added and stirred vigorously. After 30 min, TEP and CN were added successively and stirred for 3 h at room temperature. The molar ratio of BGN was 60 SiO_2: 36 CaO: 4 P_2O_5. The product was centrifuged, washed with ethanol and water for 3 times. The BGN was lyophilized and sintered at 650 ^{o}C in air for 3 h.

BGN was decorated with PDA by oxidation and polymerization of DA on its surface[4-7]. Typically, 20 mg BGN was washed with Tris for 3 (10 mM, pH 8.5) times and then dispersed in 40 mL Tris buffer. 80 mg dopamine was added to the BGN solution and stirred at room temperature for 1 h to obtain PDA-decorated BGN (BGN@PDA).

The chemical structures of F127-OTs, F127-Phe-CHO and FEPL copolymer were characterized by ^{1}H NMR spectroscopy (AvanceTM 400, Bruker, Germany) and Fourier transformation infrared (FTIR) spectroscopy. FTIR was performed on a Nicolet Nexus 670 FTIR spectrometer using the KBr pellet method in the range between 4000 and 400 cm^{-1}. The PDA decorated BGN were characterized by UV-Vis spectra and transmission electron microscopy (TEM, H-8000, Hitachi, Japan) at an accelerating voltage of 100 kV. The morphology and elemental mapping of the hydrogels were examined by field emission scanning electron microscope (FESEM, GeminiSEM 500, Zeiss, Germany) equipped with an energy-dispersive spectrometer (EDS). The crystalline composition and elemental analysis were determined by XRD and XPS, respectively.

The gelation time of the hydrogels was tested by up-down experiment. The polymer mixture was added into a vial with a diameter of 2 cm and then placed it at 37^{o}C. The gelation time was determined as the time that the mixture stopped flowing when turning the test tube up and down.

1.3. In vivo photothermal tumor therapy

All animal experiments procedures were performed according to the protocols approved by the animal care committee of Xi'an Jiaotong University. The tumor model was established by subcutaneous injection with A375 cells ($5 \times 10_6$ cells in 100 μL PBS) in the back region of each BALB/c nude mice (female, 5 weeks old). After tumor inoculation for 7 days (tumor volume $\approx$ 200 mm_3), the mice were randomly divided into four groups (n = 5) as follows: (i) FCB+NIR group, (ii) FCE+NIR group, (iii) PBS+NIR group,

(iv) FCE without laser group (FCB-NIR). Then, mice were subcutaneously injected near the tumor of PBS, FCE (100 μL, 25 wt% FEPL per mouse) and FCB (100 μL, 25 wt% FEPL and 1 wt% BGN@PDA per mouse) every other day for a total of 2 times. For the laser treatment groups, each mouse was exposed to the 808 nm laser (1.41 $W \cdot cm_{-2}$, 10 min, one time) for 3 consecutive days (from day 0 to day 2). The tumor surface temperature and thermal images were monitored and recorded by an infrared thermal imaging system. The tumor sizes were measured by a caliper every 2 days and calculated as follows: volume (mm^3) = 1/2 × length × $width^2$.

1.4 Antibacterial activity evaluations in vitro and in vivo

Typically, bacteria were cultivated in Mueller-Hinton broth (MHB) at 37 ^oC under continuous shaking at 200 rpm to mid log phase and then diluted for further use. 400 μL of FCB hydrogel, FCE, BGN@PDA and ampicillin were prepared in 24-well cell plate and incubated for 24 h at 37^oC as previously described. Subsequently, 10 μL of the stock bacteria solution (10^6 CFU mL^{-1}) in MHB was added to the surface of hydrogels. The bacteria were incubated on the hydrogel for 2 h at 37 °C after which the bacterial suspension were diluted 100 times with PBS and then 10 μL suspension were taken out and plated on a LB agar (Sigma) for another 18 h culture. The viable bacteria were observed and presented by the Colony-Forming Units per mL (CFU/mL).

The in vivo antibacterial assay was conducted on a MRSA-infected mouse model, all animal experimental procedures were conducted based on the Guideline for animal experimentation with the approval of the animal care committee of Xi'an Jiaotong University. Briefly, the MRSA-infected mouse model was constructed by subcutaneous injection of 100 μL of MRSA (1×10^8 CFU mL^{-1}) solution. After 3 days infection, 100 μL of FCB, FCE, BGN@PDA or Ampicillin (20 μg μL^{-1}) in PBS solution was injected into the infectious site once a day for total 2 times. Mice treated with PBS buffer were used as a

negative control. One day after the last treatment, all mice were sacrificed and infection tissues were excised for immunohistochemistry analysis. To measure the amount of the bacteria in the infection tissues, half of tissues were homogenized and diluted with PBS to a proper ratio, and then were plated on LB agar to analyze viable bacteria. The other half of tissues were processed routinely into paraffin, stained with hematoxylin and eosin (H&E) and observed under a light microscope.

Figures

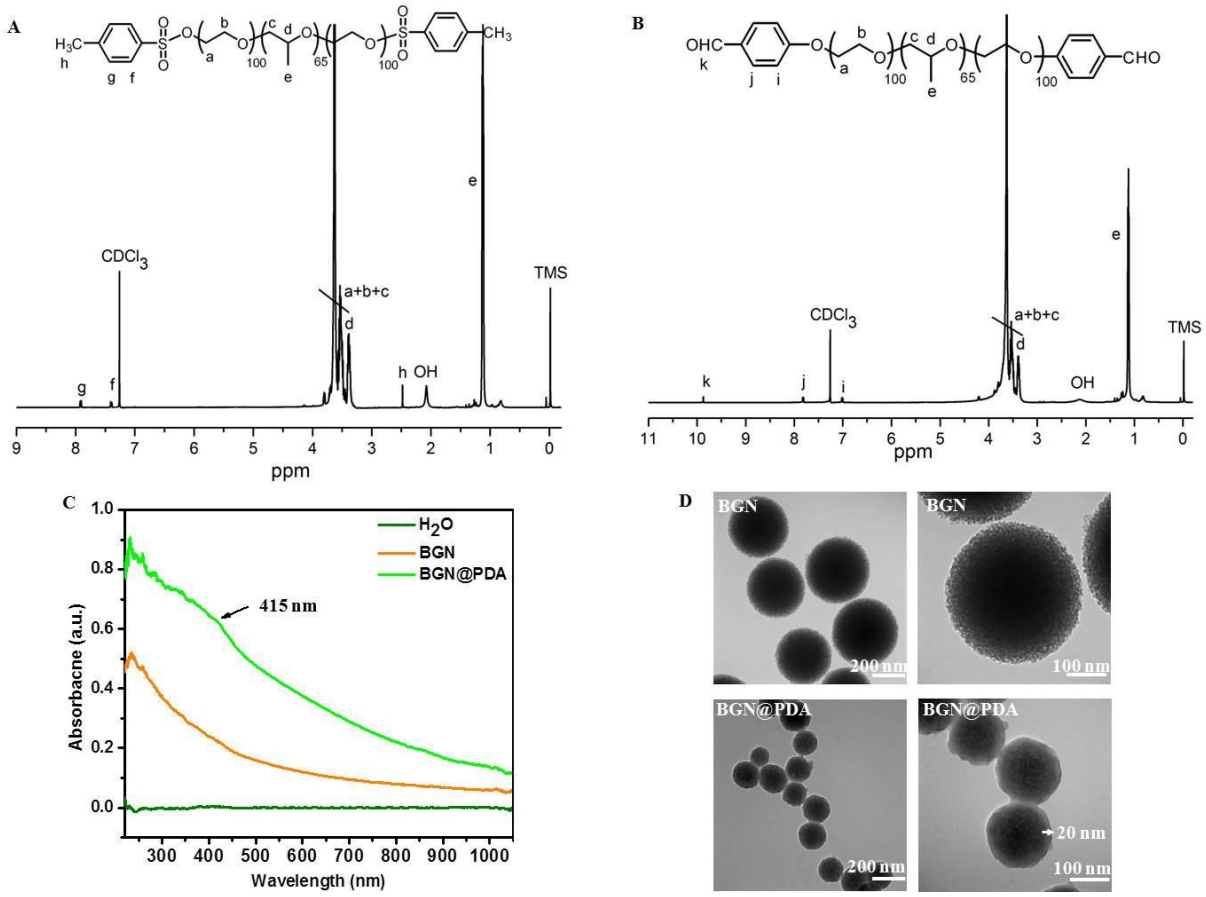

Fig. S1**.** Chemical structure analysis of prepolymers. (A-B) ^{1}H NMR spectra of F127-Ots (A) and F127-Phe-CHO (B) in $CDCl_3$; (C) UV-spectra of BGN@PDA; (D) TEM images of BGN and BGN@PDA.

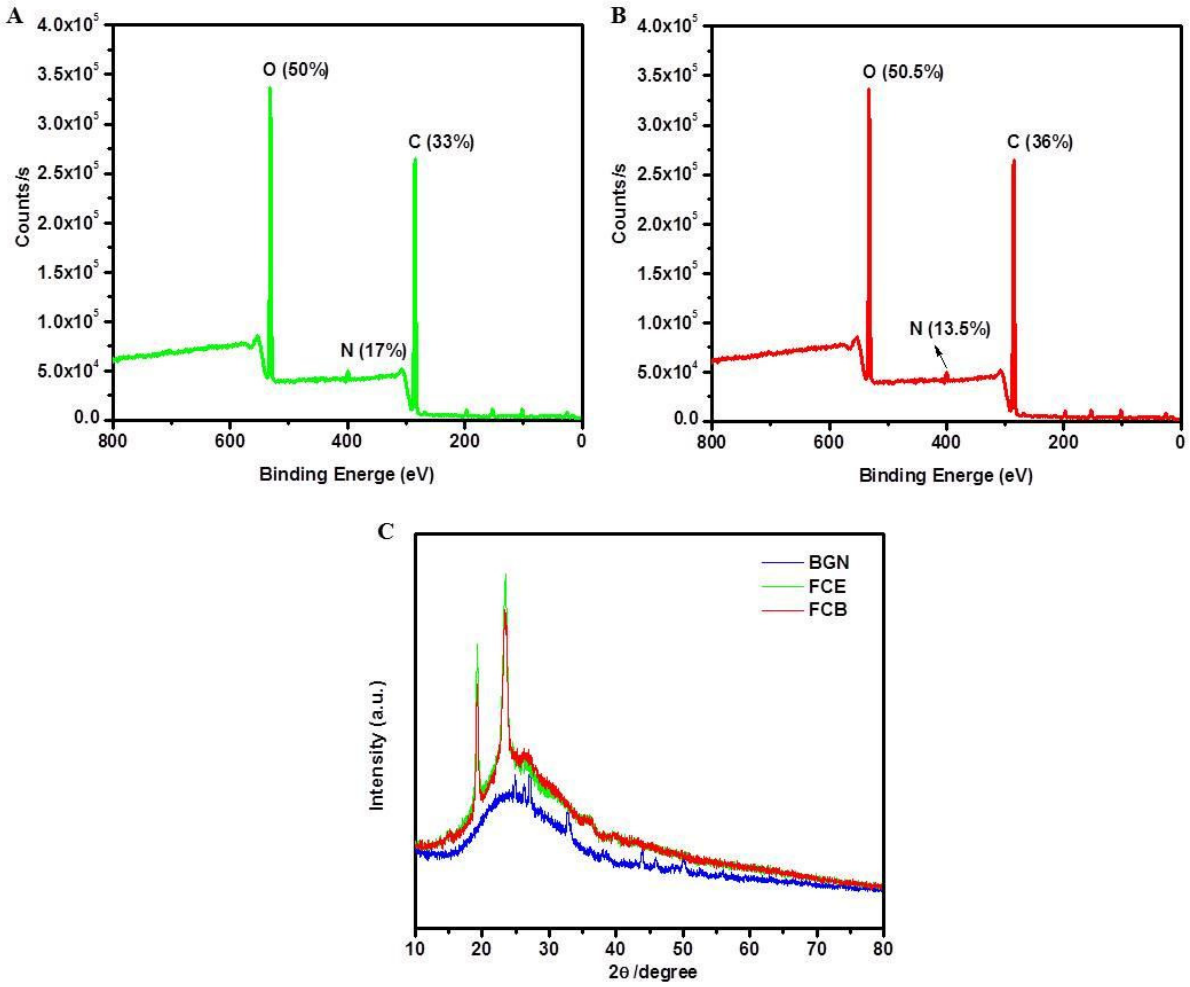

Fig S2. (A-B) XPS analysis of FCB and FCE; (C) XRD analysis of FCB hydrogel and controls.

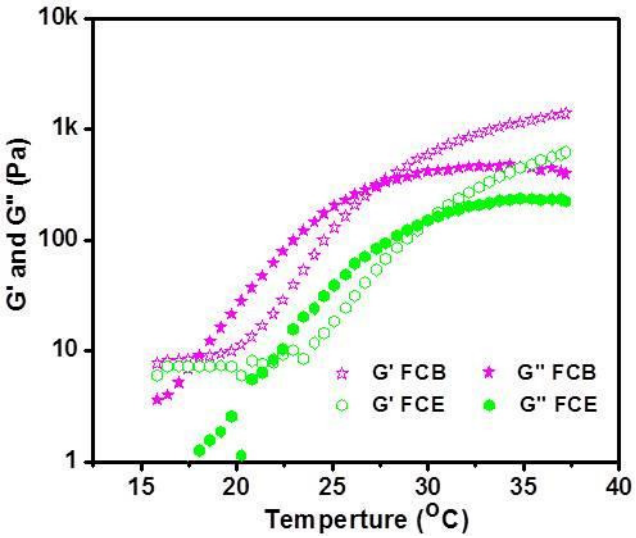

Fig S3. Rheological analysis of FCB hydrogel and controls at different temperatures

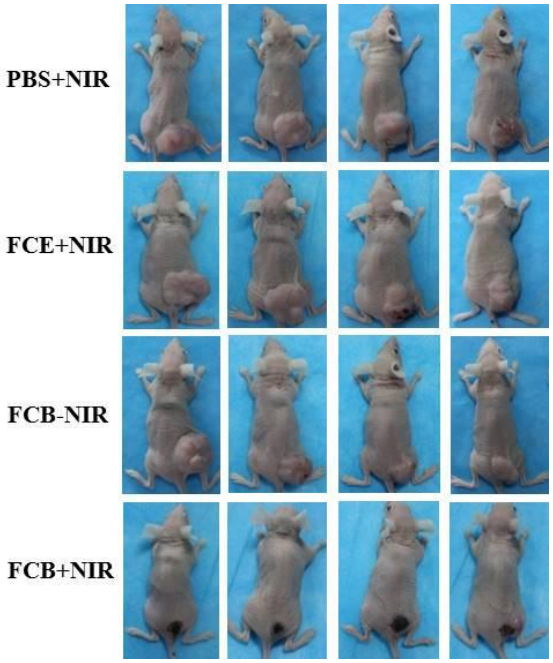

Fig S4. Representative photographs of mice after various different treatments indicated taken on days 18.

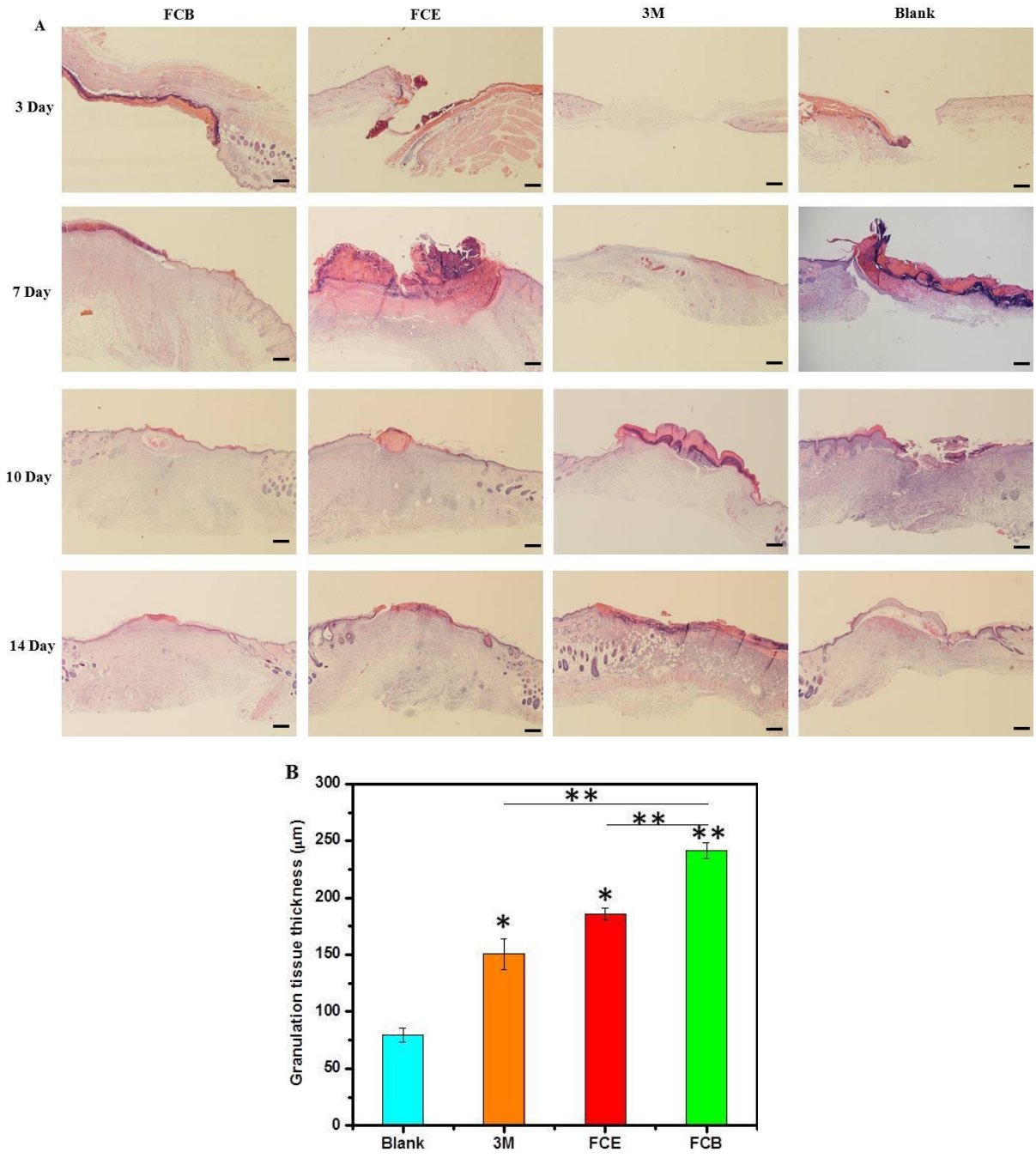

Fig S5. (A) H&E staining of wounds after 3, 7, 10 and 14 d postsurgery treated with FCB hydrogel and controls; (B) Granulation tissue thickness for FCB hydrogel group and controls at 14 d, *P < 0.05, **P < 0.01.

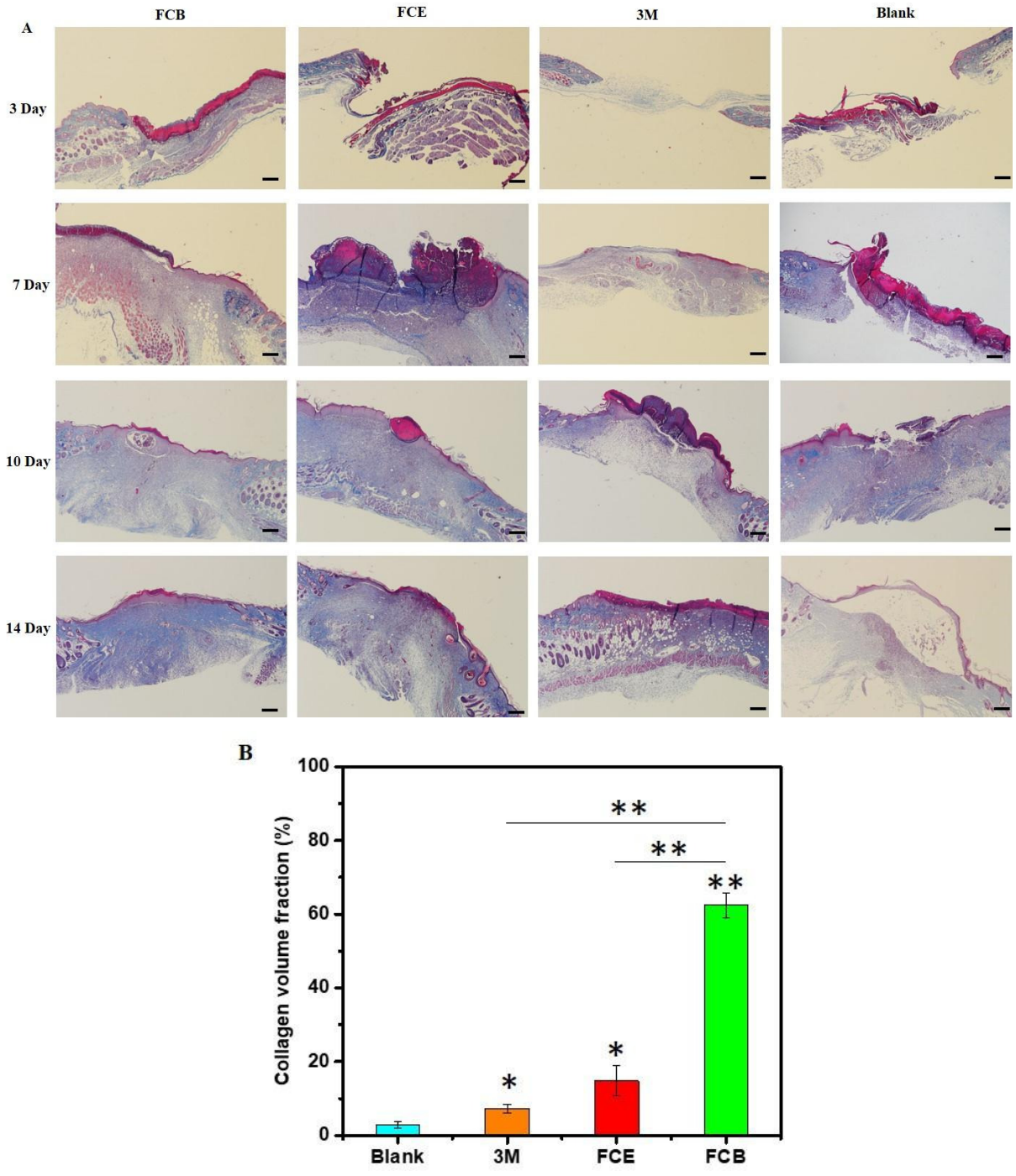

Fig S6. (A) Masson's trichrome staining of wounds after 3, 7, 10 and 14 d postsurgery treated with FCB hydrogel and controls; (B) Collagen volume fraction (%) in FCB hydrogel group and controls at 14 d, *P < 0.05, **P < 0.01, Scale bar: 200 μm.

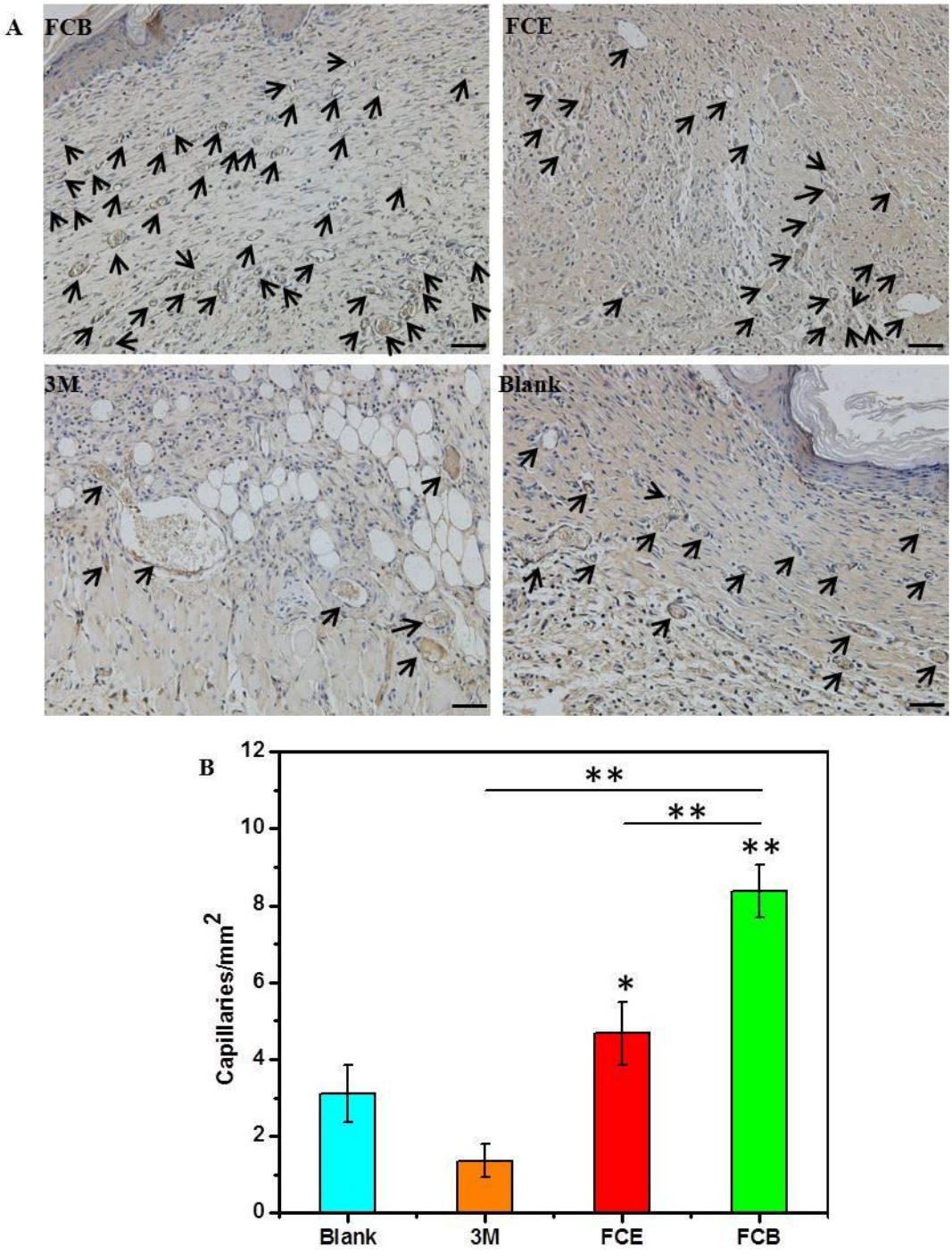

Fig S7. (A) Immunohistochemical staining of wounds after 14 days after treatment; (B) Capillaries in FCB hydrogel group and controls in wound beds at 14th day. *P < 0.05, **P < 0.01, Scale bar: 50 μm.

Reference:

1. Liu, T.; Zhang, X.; Ke, B.; Wang, Y.; Wu, X.; Jiang, G.; Wu, T.; Nie, G., F-127-PEI Co-Delivering Docetaxel and TFPI-2 Plasmid for Nasopharyngeal Cancer Therapy. Mater. Sci. Eng. C. Mater. Biol. Appl. **2016,** 61, 269-277.

2. Yang, X.; Liu, G.; Peng, L.; Guo, J.; Tao, L.; Yuan, J.; Chang, C.; Wei, Y.; Zhang, L., Highly Efficient Self-Healable and Dual Responsive Cellulose-Based Hydrogels for Controlled Release and 3D Cell Culture. Adv. Funct. Mater. **2017,** 27, 1703174.

3. Xue, Y.; Du, Y.; Yan, J.; Liu, Z.; Ma, P. X.; Chen, X.; Lei, B., Monodisperse Photoluminescent and Highly Biocompatible Bioactive Glass Nanoparticles for Controlled Drug Delivery and Cell Imaging. J. Mater. Chem. B **2015,** 3, 3831-3839.

4. Han, L.; Liu, K.; Wang, M.; Wang, K.; Fang, L.; Chen, H.; Zhou, J.; Lu, X., Mussel-Inspired Adhesive and Conductive Hydrogel with Long-Lasting Moisture and Extreme Temperature Tolerance. Adv. Funct. Mater. **2018,** 28 (3).

5. Larranaga, A.; Ramos, D.; Amestoy, H.; Zuza, E.; Sarasua, J.-R., Coating of bioactive glass particles with mussel-inspired polydopamine as a strategy to improve the thermal stability of poly(L-lactide)/bioactive glass composites. RSC Adv. **2015,** 5, 65618-65626.

6. Yu, B.; Pei, P.; Yu, B.; Li, D.; Zhang, X.; Huang, J.; Ding, H.; Chen, S.; Zhu, Y., Enhance the Bioactivity and Osseointegration of the Polyethylene-Terephthalate-Based Artificial Ligament via Poly(Dopamine) Coating with Mesoporous Bioactive Glass. Adv. Eng. Mater. **2017,** 19 (5).

7. Xu, Y.; Wu, P.; Feng, P.; Guo, W.; Yang, W.; Shuai, C., Interfacial Reinforcement in A Poly-L-Lactic

Acid/Mesoporous Bioactive Glass Scaffold via Polydopamine. Colloids. Surf. B Biointerfaces. **2018,** 170, 45-53.