Supplementary Information

Bioactive microfiber yarns serving as sutures with antibacterial and pro-tissue repair capabilities

Hao Zhang†[a], Xiaopei Zhang†[a], Manfei Fu[a], Hongshan Yang[b], Kuanjun Fang[a], Yuanfei Wang*[c], Tong Wu*[a]

[a] The Affiliated Hospital of Qingdao University, Shandong Key Laboratory of Medical and Health Textile Materials, College of Textile & Clothing, Qingdao University, Qingdao 266071, China.

[b] United Testing Services (Beijing) Technology Group Co., Ltd., Beijing 100025, China.

[c] Qingdao Stomatological Hospital Affiliated to Qingdao University, Qingdao 266001, China.

† Hao Zhang and Xiaopei Zhang have contributed equally to the work.

* Corresponding author:

Yuanfei Wang: zhizunbao19@163.com

Tong Wu: twu@qdu.edu.cn

1. Materials and methods

1.1. Materials

Polyurethane (PU), hexafluoroisopropanol (HFIP), and polyvinyl alcohol (PVA, Mw 50,000 g/mol) were provided by Macklin (Shanghai, China). Polycaprolactone (PCL, Mw 80,000 g/mol), DPPH free radical scavenging assay kit, penicillin-streptomycin, 4% paraformaldehyde (4% PFA), Dulbecco's modified eagle medium (DMEM), and 4',6-diamino-2-phenylindole (DAPI) were procured from Solarbio (Beijing, China). Phalloidin-iFluor 488 was sourced from Abcam (United Kingdom). Fetal bovine serum (FBS) was procured from Pan (Germany). Cell counting kit-8 (CCK-8) was obtained from NCM Biotech (Suzhou, China). The Calcein-AM/PI staining kit was procured from Keygen Biotech (Jiangsu, China). The mouse embryonic fibroblasts (NIH3T3) were kindly provided by the Medical Cosmetic Centre of The Affiliated Hospital of Qingdao University (Qingdao, China). Human umbilical vein endothelial cells (HUVECs) were obtained from the Advanced Biomaterials and Regenerative Medicine Group of Qingdao University. The reagents quercetin (Q), tannic acid (TA), dimethyl sulfoxide (DMSO), and sodium pentobarbital were procured from Sigma-Aldrich (USA). Agar powder, beef meal, peptone and other reagents were purchased from Obstar Biotechnology (Beijing, China). Hematoxylin & Eosin (H&E) and Masson’s Trichrome Staining Kit were purchased from Solarbio (Beijing, China).

1.2. Preparation of yarn samples

PCL and PU were added to HFIP in a 4:1 mass ratio to prepare a mixed solution with an initial concentration of 15 wt%. The yarns were prepared using a conjugate electrospinning equipment (Beijing Tianke Technology Co., Ltd., China), whereby the electrospinning solution was pumped at a rate of 0.15 mL/min with an applied voltage of 8.7 kV. A winding roller speed of 400 rpm, a collecting roller speed of 7 rpm, and a horizontal motion speed of 150 mm/min were employed in the experiment.

At the commencement of the experiment, the solution bottle was placed in a water bath and heated to 90°C. The quantity of PVA powder required for the experiment was precisely measured and added to the solution bottle gradually until complete dissolution

occurred, yielding a 10% (w/v) solution. Concurrently, 100 mg of Q powder was dissolved in 1 mL of DMSO, yielding a 10% (w/v) Q solution. Similarly, prior to the addition of the Q solution, 100 mg of TA powder was weighed and added to 1 mL of deionized water. The mixture was then stirred to obtain a 10% (w/v) solution of TA. Next, 1 mL of Q solution was added to a bottle containing 1 mL of PVA solution. After allowing for complete cross-linking of the Q with the PVA for 5-10 min, 1 mL of TA solution was introduced to the PVA solution and stirred for 5 min at 90°C. Subsequently, a further 200 μL of the mixed solution was extracted, and a 100-cm-long yarn Y with a diameter of 300 microns was removed and placed into a 100-cm-long mold with a diameter of 0.4 mm for coating. After coating, the samples were washed three times with PBS and then freeze-dried.

1.3. Characterization

The yarns were sectioned transversely using liquid nitrogen. Subsequently, the surfaces and cross-sections of the yarn samples were subjected to gold spraying and imaging with a Hitachi 8230 Cold Field Emission Scanning Electron Microscope (Tokyo, Japan). The functional groups were characterized separately for each group of yarn samples using Fourier transform infrared spectroscopy (FTIR, Nicolet iS50, USA).

The mechanical properties of the yarns were assessed by analyzing the stress-strain curve, breaking strength, and elongation at break using a universal testing machine (Jinan Hengshishengda Instrument Co., Ltd.). Finally, Y, Q-Y, and Q&TA-Y samples were mounted on a fixture, and the fixture was moved uniaxially at a constant speed of 100 mm/min. Images were plotted using Origin software.

The samples were meticulously arranged on slides to ensure uniformity and flatness, thereby creating a planar surface. Their hydrophilicity and hydrophobicity were then evaluated using a contact angle meter (OCA20, DataPhysics, Germany). Yarns of identical length were excised and weighed. Thereafter, they were immersed in a phosphate-buffered saline solution. Subsequently, the water absorption capacity of the yarn samples was quantified via gravimetric analysis, measuring the change in mass

of the samples following 10 min.

A quantity of 20 mg of yarn samples from each group was weighed and added to 30 mL of a PBS solution. The samples were then incubated at 37°C and 130 rpm. On days 1, 3, 5, 7, 10, 14 and 18, the yarns were removed from the solution and dried. The mass of the yarns was then measured after the drying process had been completed.

The stability of the yarn's surface coating was assessed through a series of rigorous tests. These included placing the yarn on a background plate, applying force through rubbing with a glove, and repeatedly adhering the tape.

1.4. Antioxidant properties

The antioxidant properties of the yarns were evaluated using a DPPH free radical scavenging kit (colorimetric method) according to the manufacturer's instructions. The formula for calculating the DPPH free radical scavenging rate is provided in equation (1):

$$D_e(\%) = \frac{A_b - (A_e - A_c)}{A_b} \times 100\% \quad (1)$$

In this context, the variable "D_e" represents the DPPH free radical scavenging rate, "A_b" represents the OD value of the blank group at 515 nm, "A_e" represents the sample group, and "A_c" represents the control group.

1.5. Biosafety assessment

The biosafety of the yarn was evaluated by culturing NIH3T3 in 24-well plates. Following a 24 h incubation period, six one-centimeter-long segments of the yarn were introduced into each well and cultured with NIH3T3 for an additional 24 h. Thereafter, the yarn was extracted, and the cells were labeled with calcein and propidium iodide, in which live and dead dyes were prepared according to AM:PI:PBS=1:1.5:2000 to differentiate between live and dead cells. The assessment of cell activity was conducted on days 1 and 3 following the co-culture with the material in question, employing the CCK-8 assay as the methodology. A total of 5,000 cells were cultured per well, and the absorbance of the supernatant was quantified using a microplate reader at 450 nm. The staining of cell morphology and nuclei was conducted using Phalloidin-iFluor 488 dye

and DAPI, respectively, with a Phalloidin-iFluor 488:PBS ratio of 1:1000. A total of 1×10^4 cells were cultured in each well. Cell morphology was observed using immunofluorescence microscopy (Echo, USA). After 24 h of incubation, the yarn was co-cultured in DMEM medium containing 1% FBS. The subsequent conditioned medium was extracted and used to inoculate 12-well plates at a density of 1×10^6 cells per well. Once the cells had reached full growth, the tip of a 200 μL sterile pipette was drawn into a straight line perpendicular to the plane of the cells in the fused monolayer. Subsequently, a 1:1 mixture of conditioned medium and DMEM medium containing 1% FBS was added, and the cells were stained with calcein yellow-green. The extent of the scratch healing was quantified and evaluated using the ImageJ software.

1.6 Antibacterial properties

The antimicrobial properties of the yarns were evaluated using the oscillation method. Nutrient broth composition: 0.6 g beef meal, 1 g peptone, 200 μL deionised water. Agar plate: 0.9 g beef powder, 1.5 g peptone, 4.5 g agar powder, 300 μL deionised water. A typical colony was picked from the plate with an inoculation ring and inoculated into 20 mL of nutrient broth. The plate was incubated at 37℃ for 18-20 h with shaking at 130 rpm to obtain a bacterial suspension containing 1×10^9 to 5×10^9 CFU/mL of viable bacteria. Pipette 2-3 mL of the bacterial suspension into a test tube containing 9 mL of the nutrient broth and mix well. Pipette 1 mL into another test tube containing 9 mL nutrient broth and mix well. Pipette 1 mL into a test tube containing 9 mL PBS buffer and mix well. Pipette 1 mL into a triangular beaker containing 14 mL PBS buffer. Mix well and dilute to contain 3×10^5 to 4×10^5 CFU/mL of viable bacteria for sample inoculation. 0.15 g of yarn samples were cut and sterilized by alcohol fumigation and ultraviolet sterilization for more than 30 min. They were then added to triangular beakers containing bacterial suspensions and incubated at 24°C with shaking at 150 rpm for 18-20 h. The solution was diluted 4 times in a series of 10-fold dilutions to the appropriate dilution. The bacterial suspension was inoculated onto agar plates using the plate spreading method. The plates were subsequently inverted and incubated at 37°C for 24 h. Plates were then photographed and counted. Similarly, after 24 h of

co-culturing with the bacterial solution, the samples were removed and immersed in a 4% PFA solution for 20 min, thereby fixing the structure of both the bacteria and the yarns. The samples were then rinsed with PBS to remove excess fixative, followed by dehydration with different concentrations of ethanol (30%, 50%, 70%, 90%, and 100%), with each concentration being immersed in ethanol for 15 min. The samples were then immersed twice in 100% ethanol to ensure complete dehydration. After dehydration, the samples were freeze-dried for 24 h. Subsequently, the yarn that had been co-cultured with the bacterial solution for 24 h was removed, and the yarn samples were immersed in a 4% PFA solution for 20 min to fix the structure of the bacteria and the yarn. The samples were rinsed with PBS to remove excess fixative before dehydration with varying concentrations of ethanol (30%, 50%, 70%, 90%, and 100%), with each concentration immersed in ethanol for 10-15 min. Finally, the samples were immersed twice in 100% ethanol to ensure complete dehydration. The dehydrated samples were prepared for SEM photography for gold spraying. The calculation of antimicrobial resistance was performed using the formula (2), where E_{anti} represents the antimicrobial rate, N_c represents the number of colonies in the blank group, and N_e represents the number of colonies in the sample group.

$$E_{anti}(\%) = \frac{N_c - N_e}{N_c} \times 100\% \quad (2)$$

1.7. Blood compatibility

Haemocompatibility testing was conducted utilizing anticoagulated whole blood derived from rabbits. The erythrocyte suspension was prepared by centrifuging the sample at 2000 rpm for 5 min to achieve a 4% volume fraction. Suspensions were prepared using saline for each group of materials, and 500 μL of the resulting solution along with 500 μL of the erythrocyte suspension were placed in a 1.5 mL centrifuge tube. After 1 h of incubation, the samples were centrifuged at 2000 rpm for 5 min, and the absorbance of the supernatant was measured at 540 nm using a microplate reader. The positive control group consisted of a mixture of 500 μL of deionized water and 500 μL of red blood cell suspension. The negative control consisted of 500 μL of saline,

thoroughly mixed with 500 μL of red blood cell suspension. The haemolysis rate was calculated using the following equation (3), in which the symbol "OD_e" represents the OD value of the sample group at 540 nm. The symbol "OD_n" represents the negative control, while "OD_p" represents the positive control.

$$\text{Hemolysis rate}\,(\%) = \frac{OD_e - OD_n}{OD_p - OD_n} \times 100\% \quad (3)$$

1.8. In vivo experimentation

A total of 12 healthy male Sprague-Dawley rats, with an average weight of approximately 380 g, were procured from the Jinan Pengyue Laboratory Animal Centre. The feeding conditions of the animals were in accordance with the standard protocol. The experimental operation was standardised, and euthanasia was performed by cervical dislocation. All experiments in this study were conducted in accordance with the relevant laws and guidelines and approved by the Animal Ethics Review Committee of The Affiliated Hospital of Qingdao University (Approval number: AHQU-MAL20240621ZH). Prior to the experiment, all suture samples were sterilised using ethylene oxide. After anaesthesia and shaving, a 1 cm incision was made on each side of the rat's back with a sterile blade. The dorsal wounds were closed using Y, Q&TA-Y and commercial suture (S). The rats were kept in cages and observed daily throughout the experiment. Images of the wounds were collected on days 0, 3, 5, 7 and 14, respectively.

On days 7 and 14, wound skin tissues were obtained from each group of rats and fixed with 4% PFA. The fixed tissues were then dehydrated in a gradient, the tissue blocks were embedded in paraffin, and the wax blocks were sectioned. The wax was then deparaffinised on two occasions, each time for 5-10 min, using xylene. Thereafter, the wax was rehydrated in gradients of 100%, 95%, 85% and 75% ethanol for 3 min each. The tissue was then immersed in distilled water for 2 min. Hematoxylin staining solution was used for 2-20 min, after which the tissue was washed with deionised water. The differentiation solution was applied for 10-60 s, after which deionised water was added dropwise or the tissue was dipped and washed twice, with each cycle lasting 3-5

min. Eosin stain was then added dropwise for 30-120 s, after which any excess stain was poured off and the tissue was dehydrated quickly. The dehydration, transparency, and sealing process involves gradient dehydration using ethanol (75%, 85%, 95%, and 100% ethanol) with each immersion lasting 2-3 s, a 1-min immersion in 100% ethanol twice, xylene transparency twice for 1 min each, and neutral gum sealing. Microscopic observation is then conducted.

Following dewaxing and washing, Weigert staining solution A and Weigert staining solution B were mixed in a 1:1 ratio and added dropwise to cover the sections for staining for 5 min. The excess staining solution was then washed off with distilled water. Afterward, an acidic differentiation solution was added dropwise to differentiate for 5-15 s. The sections were then washed in distilled water for 30 s. Subsequently, Masson's blue staining solution was added dropwise to restore the blue colouration for 3-5 min, after which the sections were washed in distilled water for 30 s. Finally, Reichhorn red magenta staining solution was added dropwise to stain the sections for 5-10 min. During the operation, the weak acid working solution was prepared according to a 2:1 ratio of distilled water to weak acid solution. This solution was then added dropwise and washed for 30 s. The excess liquid was then poured off, and the solution was dropwise added to phosphomolybdic acid solution to treat for 1-2 min. In the subsequent stage of the process, the weak acid working solution should be prepared in a 2:1 ratio of distilled water to weak acid solution. Thereafter, the weak acid working solution should be added dropwise, and the mixture should be washed for 30 s. The excess liquid should then be poured off, and phosphomolybdic acid solution should be added dropwise to treat for 1-2 min. Thereafter, the weak acid working solution should be added dropwise, and the mixture should be washed for 30 s. Aniline blue dyeing solution should then be added dropwise to stain for 1-2 min. Finally, the weak acid working solution should be added dropwise, and the mixture should be washed for 30 s. The same dewatering and sealing processes should then be carried out.

Following dewatering and cleaning, the material was restored with 6% acetic acid for 20 min, cooled to room temperature, and then cleaned. H_2O_2 was then incubated for 10 min at room temperature in the dark and washed 3 times with PBS. Drops of 5%

BSA or normal serum were then added, and the material was incubated for 30 min at room temperature. The primary antibody (CD31, 1:2000) was then added dropwise, and the reaction mixture was incubated overnight at 4°C in a wet box. Alternatively, the primary antibody was incubated at 37°C for 1-2 h. The reaction mixture was then washed 3 times with PBS (5 min each time). The secondary antibody (biotin-labelled) was then added dropwise, and the reaction mixture was incubated at room temperature for 30 min. The reaction mixture was then washed 3 times with PBS. Subsequently, the HRP-streptavidin should be added dropwise, and the incubation should be carried out at room temperature for 20 min. The washing step should then be performed three times with PBS. The DAB working solution should then be added dropwise, taking care to ensure that it is protected from light. The colour development time should be controlled under the microscope, and a positive signal should be observed to be brownish yellow. The colour development should be terminated with distilled water.

1.9. Statistical analysis

All experiments employed samples with a minimum of three replicates. The data are presented as mean ± standard deviation. A Student's t-test was employed for all two-by-two comparisons. Statistically significant results were indicated by values of $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$.

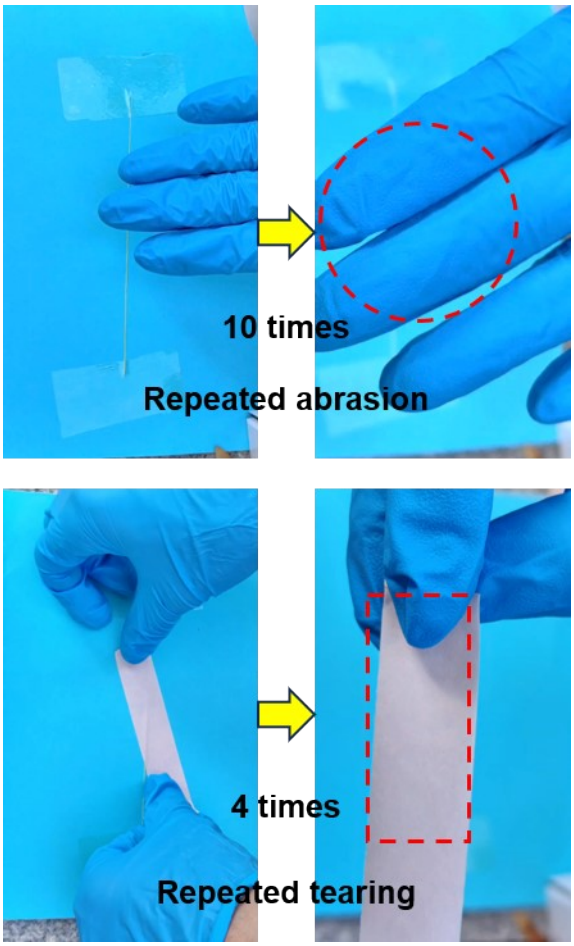

Fig. S1 The present study investigates the stability of yarn surface coatings through the repeated rubbing of gloves against tape.

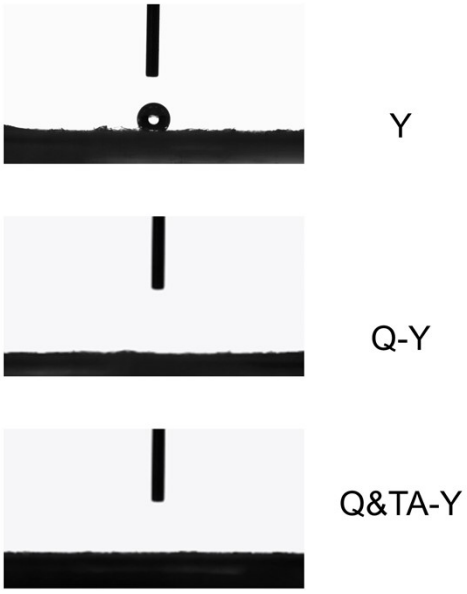

Fig. S2 Contact angle images of Y, Q-Y, and Q&TA-Y yarns.

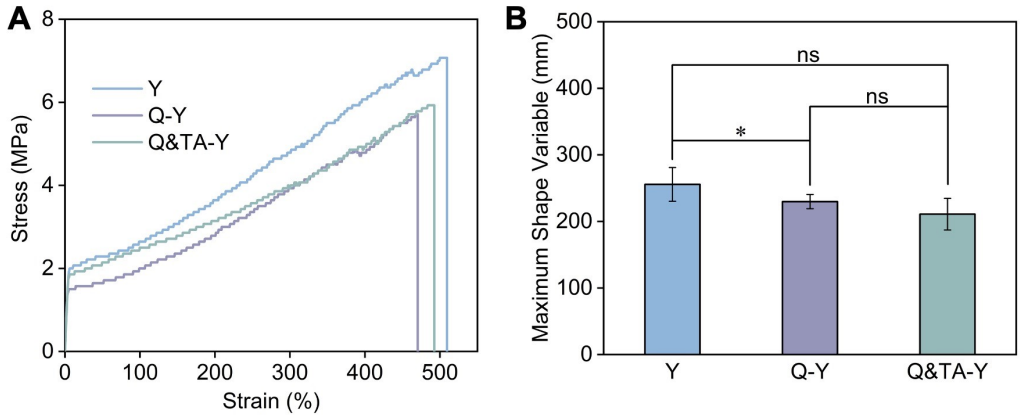

Fig. S3 A. Representative stress-strain curves obtained by tensile testing for three groups of yarns. B. Maximum stretch of Y, Q-T, and Q&TA-Y. ns $P > 0.05$, *$P < 0.05$ compared to each group.

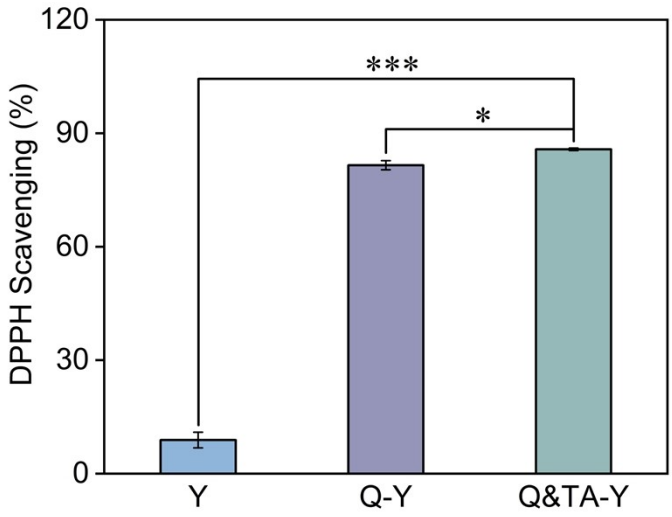

Fig. S4 DPPH radical scavenging in Y, Q-Y, and Q&TA-Y yarns. *$P < 0.05$ and ***$P < 0.001$ compared to the Q&TA-Y group.

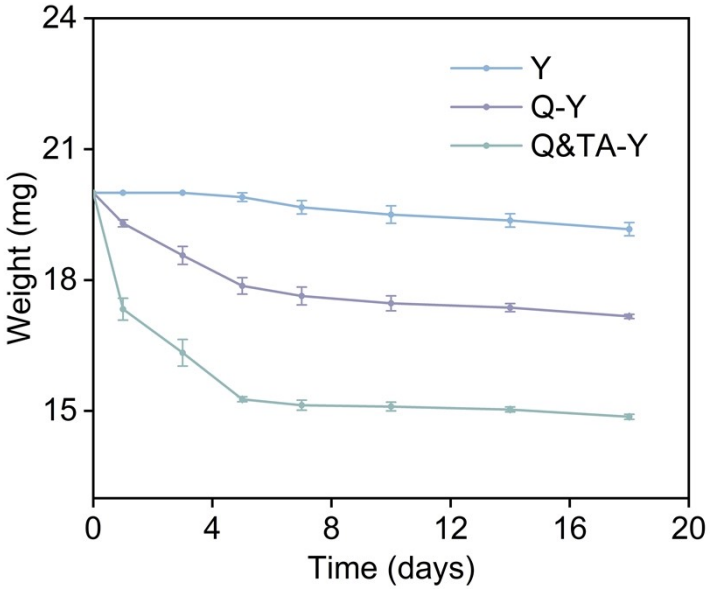

Fig. S5 The changes in the quality of the three groups of yarns in PBS at different time intervals.

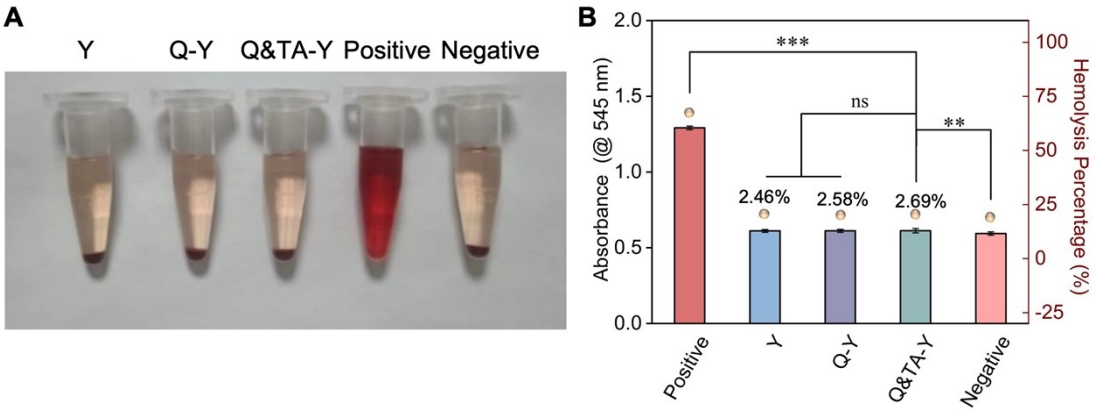

Fig. S6 A. The images of haemolysis tests in Y, Q-Y, and Q&TA-Y. B. Haemolysis rate (The yellow ball indicates the magnitude of haemolysis).

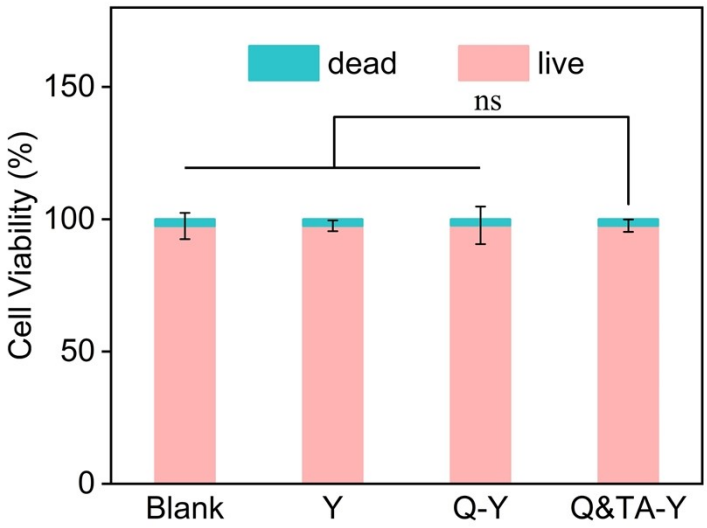

Fig. S7 Quantitative live-dead counts of NIH3T3 cells after 24 h of co-culture with Y, Q-Y, and Q&TA-Y, respectively.

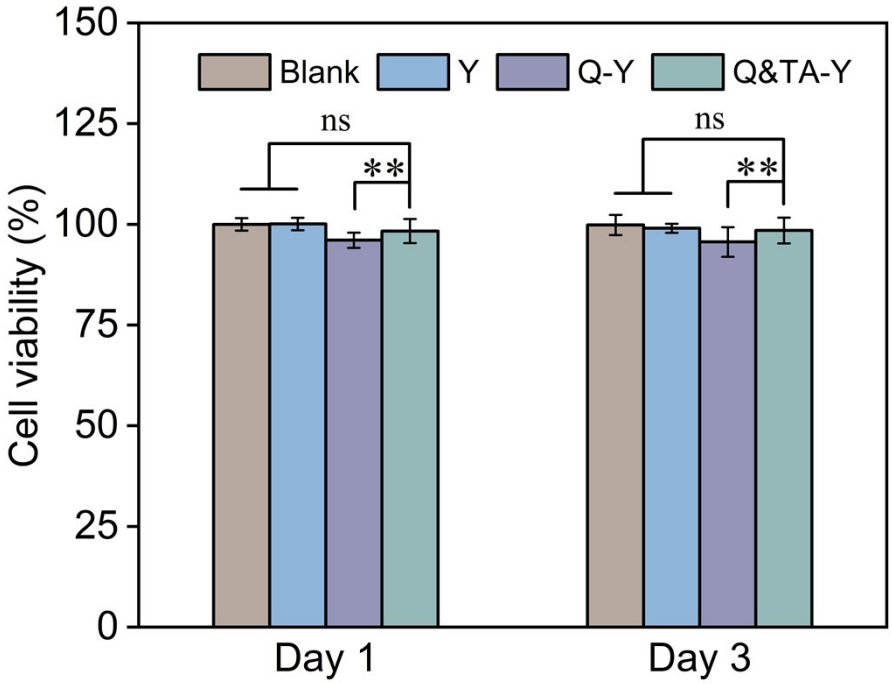

Fig. S8 Cell activity of NIH3T3 co-cultured with Y, Q-Y, and Q&TA-Y for 1 and 3 days. ns $P > 0.05$ and $^{**}P < 0.01$ compared to the Q&TA-Y group.

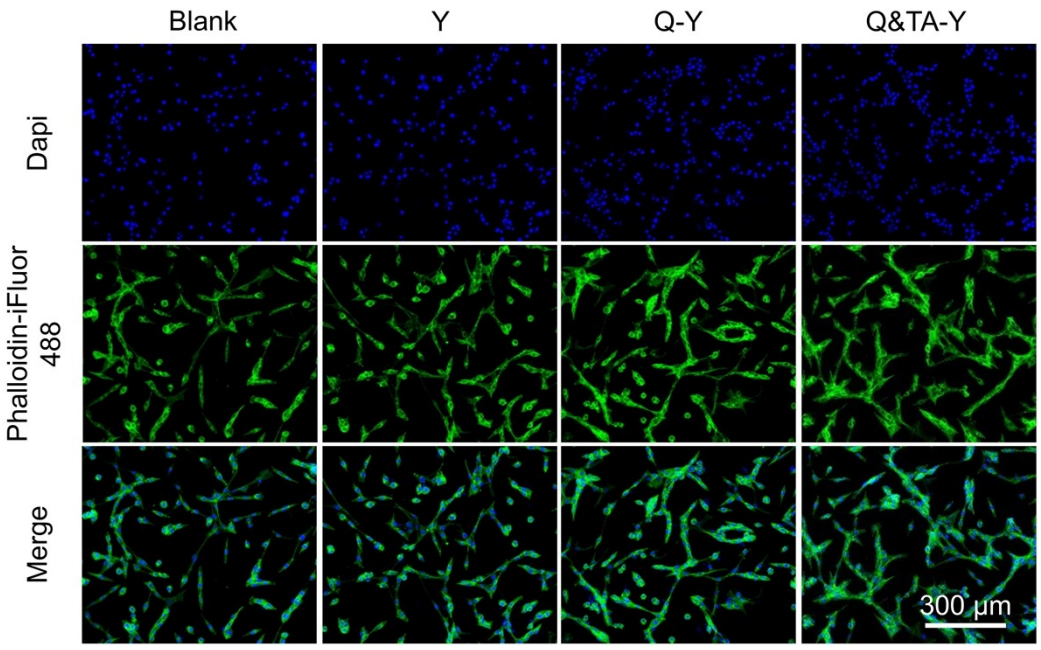

Fig. S9 Fluorescence micrographs showing the morphology of NIH3T3 at 24 h post-culture on Y, Q-Y and Q&TA-Y yarns. The nucleus and actin cytoskeleton were stained with DAPI (blue) and phalloidin-Fluor 488 (green).

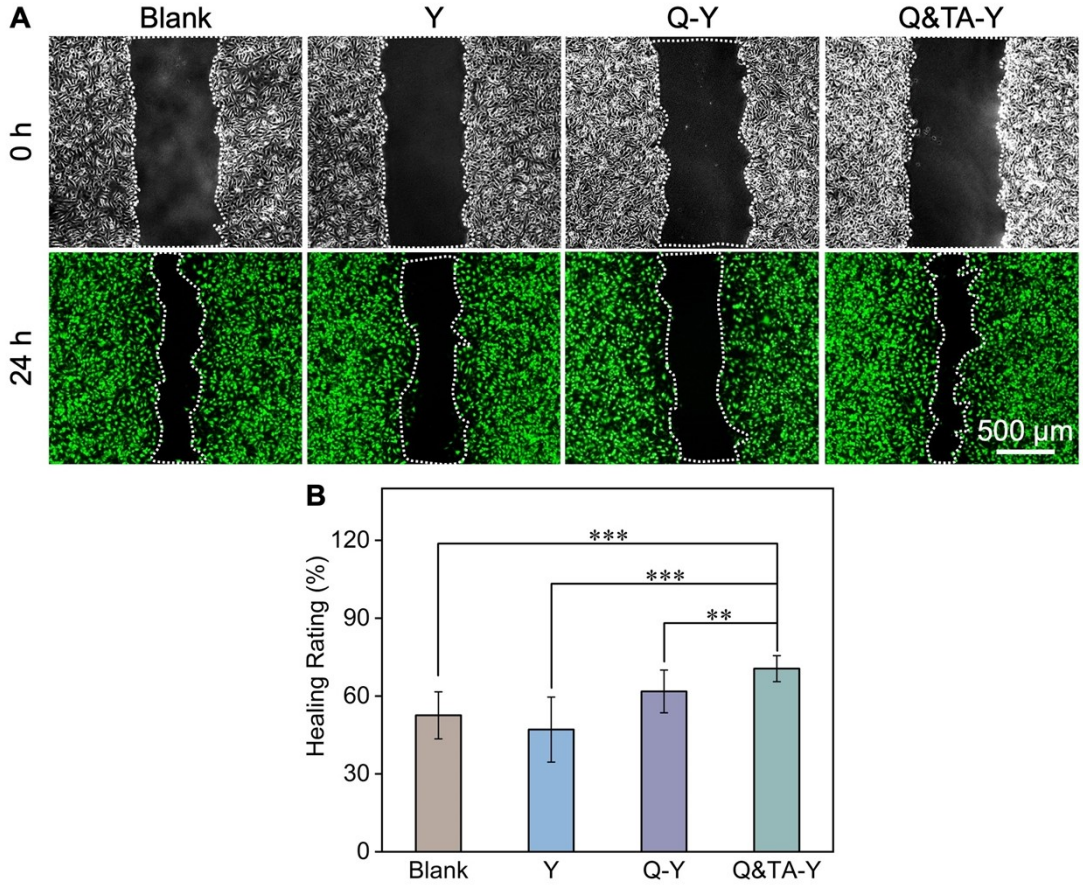

Fig. S10 A. Scratch test images of HUVECs at 0 h and 24 h in different groups. B. Healing rate of HUVECs after 24 h of co-culture with different conditioned media. $^{**}P < 0.05$ and $^{***}P < 0.001$ indicate significant differences between the Q&TA-Y group and the other groups.

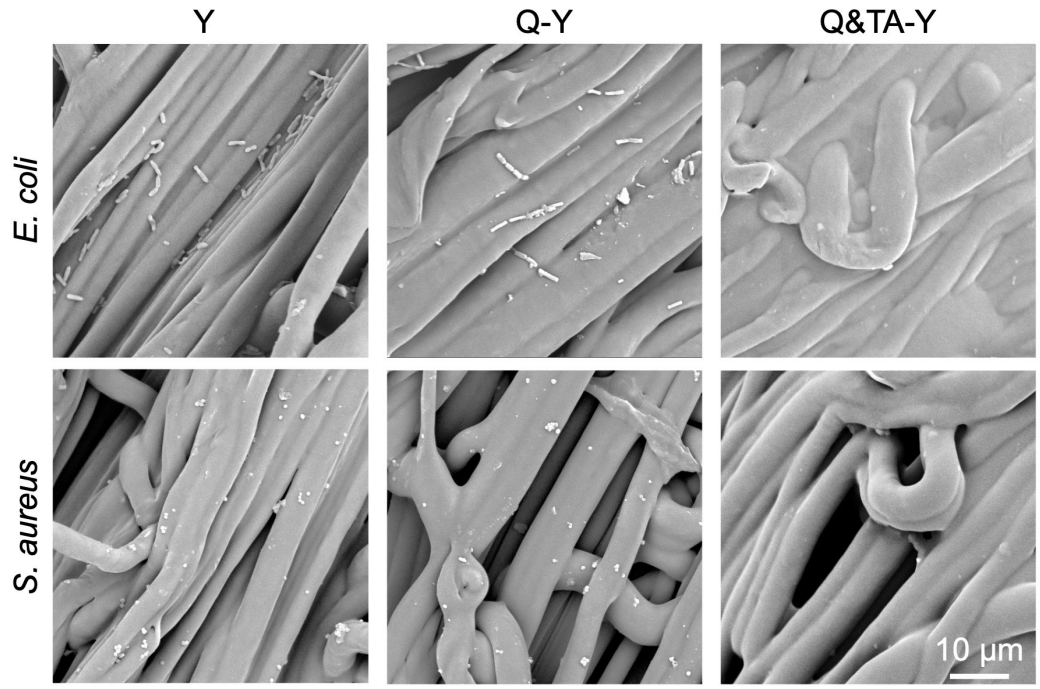

Fig. S11 Images of *E. coli* and *S. aureus* after co-culture with Y, Q-Y and Q&TA-Y (5000 ×).