Electronic Supplementary Material (ESI) for Materials Horizons.

Supporting Information

Close-loop Dynamic Nanohybrids on Collagen-Ark with In-situ Gelling Transformation Capability for Bio-mimetically Stage-Specific Diabetic Wound Healing

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KEYWORDS: phenylboronic acid ester; porous silicon; nanohybrids; collagen; diabetic wound healing

Experimental Section:

Materials and Cell Culturing

Type I water insoluble collagen patch was supplied by MBP GmbH, Germany. All chemicals were purchased from Sigma–Aldrich unless otherwise specified and were used as received with no further purification. The deionized water was obtained by a Millipore NanoPure purification system (resistivity > 18.2 MΩ cm^{-1}). All the organic solvents for synthesis and analysis were ordered from Fisher Scientific Inc., USA, and used as received.

Dulbecco's Modified Eagle's Medium (DMEM), Medium-200 (M200), fetal bovine serum (FBS), trypsin (2.5%), sodium pyruvate, nonessential amino acids (NEAA, 100×), L-glutamine (100×), penicillin-streptomycin (100×) were purchased from HyClone (Waltham, USA). Low serum growth supplement (LSGS) was purchased from Thermal Fisher Scientific Inc. (USA)

Synthesis of 4-(Hydroxymethyl)-Phenylboronic Acid Pinacol Ester Conjugated Oxidized Dextran (POD).

POD was prepared according to the literature with some modifications.[1] Briefly, 4-(hydroxymethyl)- phenylboronic acid pinacol ester (PAPE) (400 mg, 1.71 mmol, SigmaeAldrich, USA) was dissolved in dry dichloromethane (DCM, 2 ml) in a dried 20 ml flask at 50 °C. Carbonyldiimidazole (CDI, 554 mg, 3.42 mmol, Sigma-Aldrich, USA) was then added to the solution and stirred for 1 h. The mixture was concentrated under vacuum, re-dissolved in ethyl acetate (200 ml) and washed with H_2O (3 × 1 mL). The organics were dried with $NaSO_4$, and concentrated using a rotary evaporator to give a pure transparent oily PAPE-CDI. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.27 (s, 12 H), 4.54 (s, 2 H), 7.04 (s, 2H), 7.35 (d, 2H), 7.65 (d, 2H), 7.71 (s, 1H).

Dextran (1 g, average Mw 9000−11000 Da; Sigma-Aldrich, USA) was dissolved in 18 ml water. After adding sodium periodate (0.855 g, Sigma-Aldrich, USA), the solution was stirred for 5 h at room temperature (RT). The product was purified by dialysis of the solution against distilled water for 48 h using a regenerated cellulose membrane with MwCO of 3500 Da. Then the sample was lyophilized to obtain oxidized dextran (ODEX).

The obtained ODEX was further used for PAPE modification. Briefly, 90 mg as-prepared ODEX was totally dissolved in 1.2 ml of anhydrous dimethyl sulfoxide (DMSO), PAPE-CDI (1 g, 3.01 mmol) was added followed by 4-dimethylaminopyridine (DMAP, 240 mg, 1.96 mmol) addition, and the mixture solution was stirred for 20 h at 50 °C. The product POD was isolated by centrifugation at 16000*g* for 5 min then wash with dd-H_2O (3 × 10 ml). The residual water was removed with lyophilization, producing POD (250 mg) as a white solid. The degree of functionalization was determined by ^{1}H NMR and the final PAPE functionalization degree is around 31%. ^{1}H NMR (400 MHz, DMSO-d_6): 1.29 (s, pinacol), 4.06–4.17 (br, oxidized-dextran), 5.13 (s, ethyl), 5.16–5.23 (br, oxidized-dextran), 7.35, 7.70 (d, phenol).[2]

Preparation of Porous Silicon Nanoparticles (PSi).

The fabrication of PSi nanoparticles has been described elsewhere.[3] Briefly, the formation of the porous structure was made using monocrystalline, boron doped p+ Si ⟨100⟩ wafers with a resistivity of 0.01–0.02 Ω·cm with a 1:1 (v/v) hydrofluoric acid (38%)–ethanol electrolyte, by electrochemical anodization. The application of pulsed low/high etching current repeatedly provided a multilayer structure on the Si wafers, with high porosity fracture planes for nanoparticle generation. By increasing the etching current abruptly, the multilayer film was lifted off from the substrate. The multilayer PSi films were stabilized by thermal hydrocarbonization (THCPSi), under exposure to a N_2/acetylene (1:1) flow at 500 °C, and further functionalization of the THCPSi film was performed by a thermal treatment in undecylenic acid (PSi) for 16 h at 120 °C. The PSi films were then wet milled in a 5 vol. % undecylenic acid–dodecane solution. The desired particle size was obtained by centrifugation.

Determination of Oxidation Degree of ODEX.

The oxidation degree of ODEX was determined by quantifying the aldehyde groups formed by ethyl carbazate via carbazone formation. Briefly, ODEX solution (10 mg ml^{-1}) was prepared in acetate buffer with the pH value of 5.2, followed by adding two-fold of ethyl carbazate and was allowed to react for 24 h at RT. Afterwards, 2 folds of $NaBH_3CN$ was added and reacted for another 12 h. The product was dialyzed thoroughly against water for 24 h followed by lyophilization. The degree of oxidation (i.e., abundance of aldehyde groups) was assessed by ^1H NMR by integrating the peaks at 1.2 ppm (methyl groups) against 4.7 ppm (anomeric proton from dextran). And the results suggested the oxidation degree of ODEX obtained was 40.2%.

Degradation Behavior of POD.

We first used light scattering study to investigate the polymer degradation.[1b, 4] Hydrolysis of PAPE-ODEX was performed in dd-H_2O and the same PBS containing various concentrations of H_2O_2. The absorbance of each sample was measured at 500 nm (Thermo Fisher Scientific, USA). This absorbance corresponds to scattered light from the particles, whereas POD itself does not absorb at 500 nm. After each measurement, the particles were collected by centrifuge (16000*g*, 5 min) and re-suspend in the fresh solution with corresponding H_2O_2 concentration. The degradation was calculated as follows:

$$\% \text{ degradation} = 100 * [1-(A-A_\infty)/(A_C-A_\infty)],$$

where, A is absorbance of each sample, A_C is absorbance of control group at each time point, and A_∞ is absorbance of PBS. The curve was fit to one phase exponential decay (dissociation kinetics).

^1H NMR was also conducted to better understand the mechanism of the degradation. POD was suspended in D_2O (0.6 ml) with or without 1 mM H_2O_2 for 6 h. Then the solution was centrifuged to get rid of the un-dissolved polymer and further transferred to NMR tube for further measurement.

Glucose Oxidase (GOx) Induced Glucose Dependent pH Drop and H_2O_2 Generation.

High glucose (4 mg ml^{-1}) dulbecco's modified eagle medium (DMEM, Sigma-Aldrich, USA) containing phenol red was used as pH indicator to investigate the GOx induced pH drop. DMEM containing different concentrations of GOx was incubated at 37 °C and the absorption at the wavelength of 560 nm was measured with a Varioskan Flash fluorometer (Thermo Fisher Scientific, USA) at each time point.

2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) method was used to evaluate the H_2O_2 generation caused by GOx. 0.01 M of PBS buffer (pH 7.4) containing 0, 1 or 4 mg ml^{-1} glucose were prepared followed by adding same amount of GOx. Samples were collected at each time point to measure the H_2O_2 concentration within the solution. 200 μM of DCFH-DA was hydrolyzed in 8 mM NaOH solution for 30 min to yield DCFH. Afterwards the as-collected samples were stained with 10 μM of DCFH for 10 min, then analyzed with Varioskan Flash fluorometer (Thermo Fisher Scientific, USA) at the excitation and emission wavelength of 488 nm and 522 nm, respectively.

Fabrication of Dual-drugs Co-loaded Nanohybrids.

POD can not fully dissolve in preferred solvent, therefore prior to the experiment, we dispersed 30 mg of POD into 1 mL EtOH/H_2O co-solvent (80/20, v/v), after batch sonicating for 10 min, the sample was centrifuged and the supernatant was collected with the POD concentration of 15 mg ml^{-1}. For the preparation of PSi encapsulated nanohybrids (PSi@POD), microfluidic 3D co-flow focusing glass capillary device was used at RT.[5] PSi (1 mg ml^{-1}) was dispersed into the POD (15 mg ml^{-1}) ethanol/H_2O (80/20, v/v) co-solvent solution with a desired concentration, which served as the inner fluid together; the outer fluid was composed by dd-H_2O (pH = 7.2). The inner (1 ml h^{-1}) and outer (10 ml h^{-1}) fluids were separately pumped

into the microfluidic device, in which the inner fluid was focused by the outer continuous fluid. In this procedure, water insoluble POD self-assembled into the nanocarriers during diffusion from the organic solution into water, and thus, the nanohybrids were obtained.

For atorvastatin (ATO, TCI, Japan) loaded nanohybrids, 4 mg ml^{-1} of PSi was co-mixed with 20 mg ml^{-1} of ATO methanol solution for overnight, then the ATO-loaded particles were collected through centrifuge (16000*g*, 5 min) and use dd-H_2O to gently wash away the residual methanol on the surface. Afterwards it was thoroughly dispersed in POD solution in corresponding concentration and was doing the microfluidic-assisted nanoprecipitation, as described previously.

To construct deferoxamine (DFO), ATO dual-drugs loaded glucose responsive nanohybrids, 100 μg of DFO and 10 μg of GOx were added in 10 ml as-prepared ATO-encapsulated PSi@POD solution and stir for overnight to initiate the Schiff-base facilitated drug conjugation.

Physicochemical Characterization of the NPs.

The qualitative analysis of the surface modification of the NPs was performed by Fourier transform infrared spectroscopy (FTIR) with a Vertex 70 spectrometer (Bruker Optics, USA) using an horizontal attenuated total reflectance (ATR) accessory (MIRacle, PIKE Technologies, USA). The spectra were recorded between 4000 and 500 cm^{-1} with a 2 cm^{-1} resolution.

Hydrodynamic diameter (Z-average) and zeta-potential measurements of the NPs were carried out using a Zetasizer Nano ZS (Malvern Instruments, UK) at 25 °C.

The morphology, shape and dispersity were studied by imaging the NPs with a transmission electron microscope (TEM, Tecnai 12, USA). The EDX spectra of the PSi@POD was measured with an Oxford INCA 350 EDX (Oxford, UK) microanalysis system connected to the Hitachi S-4800 (SEM, Hitachi, Japan).

Rheology Measurement.

The rheological behavior of collagen patches with different groups were performed on a TA Instruments AR2000 rheometer with a 20 mm diameter stainless steel plate-and-plate geometry. Five different samples, including C, COD, CP, CP1 and CP4 were prepared as followings. Collagen patch was immersed in PBS buffer (pH = 7.4) to obtain group C; collagen patch was immersed into PBS buffer (pH = 7.4) with 5 mg mL^{-1} ODEX to obtain group COD; D-G-PSi@POD containing 500 μg of PSi was embedded on the collagen patch, then the patch was immersed into PBS buffer (pH = 7.4) to obtain group CP; D-G-PSi@POD containing 500 μg of PSi was embedded on the collagen patch, then the patch was immersed into PBS buffer (pH = 7.4) containing 1 mg mL^{-1} glucose to obtain group CP1; D-G-PSi@POD containing 500 μg of PSi was embedded on the collagen patch, then the patch was immersed into PBS buffer (pH = 7.4) containing 4 mg mL^{-1} glucose to obtain group CP4. All collagen patches with different groups were made to 20 mm diameter and 2 mm thick disks to facilitate the rheological measurements. The oscillatory stress sweep tests were made in strain controlled mode from 0.1 % to 100 % strain at a frequency of 1 Hz. Frequency dependencies for oscillatory shear of the different samples were made with a fixed strain of 0.1 %. All tests were performed at 33°C.

The viscoelastic properties of the materials were also compared by the viscoelastic figure of merit (VFOM). Namely, we used VFOM = $|G^*|\tan\delta$, where $G^* = (G'^2 + G''^2)^{0.5}$ and $\tan\delta = G''/G'$, where G* is the complex dynamic modulus, G′ is the storage modulus and G″ is the loss modulus. The moduli values for the calculations were taken from the linear viscoelastic region.

In Vitro Drug Release Behavior of Different Drugs.

The analytical method for both ATO and DFO were first established. High performance liquid choromtography (HPLC) method were used for ATO analysis, the HPLC system (Shimadzu, Japan) consisted of a LC-10 AT VP pump, SIL-10 AD VP sample injector, and FCV-10 AL UV/Vis detector. Chromatographic separations and subsequent quantifications of ATO were carried out at room temperature using an analytical column Discovery C_{18}, 4.6 × 150 mm (GL Sciences Inc.). The mobile phase consisted by 0.01 M of KH_2PO_4 (pH 3.75)water and ACN (20:80, v/v) were pumped at a flow rate of 1 ml min^{-1}. The mobile phase were prepared freshly and degassed by sonication for 5 min before use. The injection volume was 20 µL, and the detection wavelength was 248 nm.

A colormetric method was applied for DFO quantification. 5 µL 800 mg mL^{-1} $FeNO_3 \cdot 9H_2O$ was added into 200 µL DFO contained samples, then the samples were measured with a Varioskan Flash fluorometer (Thermo Fisher Scientific, USA) at the wavelength of 460 nm.

The H_2O_2-dependent *in vitro* release of ATO from the PSi@POD was evaluated in phosphate buffer solutions with H_2O_2 concentration of 0, 100, 250, 500, 1000 µM in sink conditions. The drug-loaded nanohybrids containing 100 µg of PSi was put into buffer solution using a shaking method at 250 rpm and 37 °C. To each time point, 100 µL volume of releasing medium was taken to centrifuge (4 min, 5000*g*) and the supernatant was collected. Then the same amount of preheated medium was added back along with the centrifuged particles to replace the withdrawn volume. The concentration of free drugs at each time point was quantified by HPLC, as described above. All the data were replicated at least 3 times.

The GOx induced glucose responsive drugs release from the nanohybrids (with or without embedded in the collagen patch) were further evaluated. We first evaluated the release behavior of the free particles. DFO/GOx conjugated PSi@POD containing 300 µg of PSi was suspended in 0.01 M of PBS buffer (pH 7.4) with 0, 1 or 4 mg mL^{-1} of glucose. Then the sample collection and measurement was the same, as described previously.

The release behavior of DFO/GOx conjugated PSi@POD embedded in collagen patch was evaluated with Transwells. The particle embedded patch was put in the upper chamber and the doner chamber was filled with corresponding PBS buffer containing different concentrations of glucose. Free drugs embedded in the patch was used as free drug control. Then the sample collection and measurement was the same as described previously.

In vitro Cytotoxicity Study.

The cytotoxicity of the different formulation groups was evaluated by an ATP-based luminescent cell viability assay, as described elsewhere.[6] Briefly, human dermal fibroblast cells were seeded in 96-well plates at the density of 2×10^4 cells per well and allowed to attach overnight. Then, the cell culture medium was replaced by 100 µl of medium (containing 10% FBS), containing different concentrations of dual drug loaded PSi@POD (10, 25, 50 and 100 µg mL^{-1} of PSi concentration) and PSi, POD, PSi@POD, D-G-PSi@POD, POD+GOx, free GOx, free ATO, free DFO and ATO/DFO combination free drug with corresponding concentrations. After 24 h and 48 h incubation, the number of living fibroblast cells was determined by the ATP-luminescent based cell viability kit (CellTiter-Glo®, Promega, USA), according to the manufacturer's protocol. Each experiment was performed at least in triplicate, and the luminescence was measured with a Varioskan Flash fluorometer (Thermo Fisher Scientific, USA).

Tube Formation Assay.

To assess the ability of DFO released from the NPs embedded patch to promote angiogenesis, growth factor-reduced Matrigel (150 µL $well^{-1}$, BD Biosciences, US) was used to induce tube formation in a 24-well Transwells.[7] The Matrigel was first mixed with ice-cold FBS free M200 medium (1/3, v/v), then add to the pre-cooled donor chamber, and shaking for 30 min at room temperature. 6×10^4 human umbilical vascular endothelial cells (HUVECs) were seeded in each well, with the free drug embedded patch or NPs embedded patch placed in the upper

compartment of a 0.4 μm Transwell (Corning, US). The cells were then incubated at 37 °C to form tubes. Tube formation was evaluated with a microscopy (5× objective, ZEISS camera).

Streptozotocin (STZ) Induced Type II Diabetic Mice Model.

Intraperitoneal (*i.p.*) injection of streptozocin (STZ, Sigma-Aldrich, US) was applied to induce mice diabetic model.[8] All experiments involving animals were performed in compliance with the guidelines from the Institutional Animal Care and Use Committee at Experimental Animal Centre in Xiamen University. Male C57BL/6 (20-25 g) mice were fed with a high fat diet containing 60% kcal for 4 weeks before the STZ- induction of diabetes. Afterwards mice were *i.p.* injected with 50 mg kg^{-1} of STZ which was freshly dissolved in 0.1 M sodium citrate buffer at the pH value 4.5. STZ was administrated once daily for 5 consecutive days to induce pancreatic islet β-cell death. All mice were fed a high fat diet during the induction process. To evaluate the successful establish of type II diabetes mice model, blood glucose of the mice was monitored by ONETOUCH® UltraEasy® glucometer, and a mouse was considered diabetic after three consecutive readings of >250 mg dl^{-1} blood glucose.

***In Vivo* Wound Healing Process.**

The diabetic mice were anesthetized with isoflurane and the hair on their backs was shaved and completely removed with depilatory cream. After disinfection with alcohol swabs, one wound was gently outlined by a marked 6 mm punch biopsy (Acuderm, Fort Lauderdale, FL). Following the outline, full-thickness wounds were made using a McPherson-Vannas Micro Scissor (World Precision Instruments, Sarasota, FL). The mice were randomly divided into 4 groups (n = 5): /1) blank PSi@POD embedded in collagen patch (CP); /2) free agents (including ATO, DFO and GOx) embedded in collagen patch (CF); /3) ATO and GOx loaded PSi@POD embedded in collagen patch (PA); and (4) ATO, DFO and GOx loaded PSi@POD embedded in collagen patch (PAD). Wounds were further covered with Tegaderm and coban, animals were individually caged, and the various treatments were reapplied every 3 days. Wounds were photographed with digital camera, wound area was calculated with Image J.

Histological Analysis.

Wounds were harvested on days 7, 14 with a 3 mm rim of unwounded skin for histological analysis. The samples were fixed in 4% paraformaldehyde overnight, embedded in paraffin and then sectioned (7 μm). The length of the new epidermis was used to evaluate the wound healing conditions after hematoxylin and eosin (H&E) staining. Ki-67 (1:200, RLM3064, Ruiying Biological, China) and VEGF (1:200, 19003, Proteintech, China) staining was used to investigate the regeneration process at days 7 and 14. Leica® DM4 Upright microscope was used for catching digital photographs and Image J software was used to evaluate the wound healing process.

Detection of Vascularization and MVD.

Specimens were embedded in optimal cutting temperature compound (OCT) and frozen in liquid nitrogen for fluorescent immunostaining. 7 μm thick frozen sections were blocked in 10% normal goat serum/0.05% Triton X-100/0.2% BSA (Sigma) in PBS for 60 min at RT. The sections were incubated overnight at 4 °C with a CD31 (PECAM-1) antibody (1:200, AF3628, R&D System, US), followed by incubation with Alexa Flur® 488nm rabbit antigoat IgG-TR (1:100, ZF0514, ZSGB-BIO, China) secondary antibody for 1 h at 37 °C. DAPI (Sigma) was used to stain nuclei for 10 min. Subsequently, sections were imaged with Zeiss LSM 780 (Carl Zeiss, Germany).

Microvessel density (MVD) was assessed in the wound bed under 200 × magnification for each specimen. Subsequently, the microvessel numbers were counted at 200× magnification within the hot spot area. Only the vessels with clear clusters of endothelial cells with lumen were counted. The MVD value was defined as the average vessel count in 5 specimens.

Western Blot Investigation.

For Western Blot analysis, fresh skin tissue samples were prepared in ice-cold lysis buffer containing 1% of Triton X-100, 50 mM of Tris-HCl (pH 7.4), 10% of glycerol, 150 mM of NaCl, 2 mM of EDTA, 50 mM of NaF, 10 mM of Na_3VO_4, complete Mini protease inhibitor mixture tablet (Roche), and 2 mM of PMSF. Total protein content was determined using a BCA protein assay kit (Beyotime, China). Lysate corresponding to 20 μg of total protein was subjected to SDS-PAGE, and transferred to polyvinylidene fluoride membrances (100 V, 90 min).

Then the proteins were identified by immunoblot analysis with the appropriate primary antibodies at a dilution of 1:1000 (or as otherwise stated below). Anti-β-actin (A1978, Sigma-Aldrich), anti-Hif-1α (10006421, Cayman Chemical), anti-VEGF (19003, Proteintech). Biotinylated secondary antibody to Rabbit IgG (BAF008 1:5000 dilution for each) was from R&D System. The protein brands were visualized with a SuperSignal West Pico Kit according to the manufacturer's instruction (Thermo Fisher Scientiftic Pierce).

Assessment of Cellular Cytokine Release by ELISA Methods.

For ELISA analysis, fresh wound samples were harvested and stored at -80 ºC, before the test, all skin samples were prepared in ice-cold saline. IL-1β IL-6 and TNF-α levels were analyzed in the same supernatants by using the mouse ELISA kit (cat. EM001, EM004, EM008, ExCell Bio®, China). All the assays were performed according to the manufacturers' instructions. Absorbance was measured at 450 nm with a Varioskan Flash spectrophotometer (Thermo Fisher Scientific, USA.), and the cytokine quantities were calculated through the use of standard curves for each recombinant cytokine.

Statistical Analysis.

Results are expressed as the mean ± standard deviation (SD) or mean ± standard error of the mean (SEM) for at least three independent experiments. Data were analyzed using the Student's *t*-test or one-way analysis of variance (ANOVA) followed by a Bonferroni's post-hoc test for multiple comparisons (SPSS 20; IBM Corp., USA).

Supporting Table

Table S1. VFOM of different hydrogels at 8 Hz.

	C	COD	CP	CP1	CP4
tanδ	0.15	0.11	0.17	0.12	0.15
VFOM	322	1045	998	880	1668

Note: 1 Hz = 6.283 rad/s

Supporting Figures

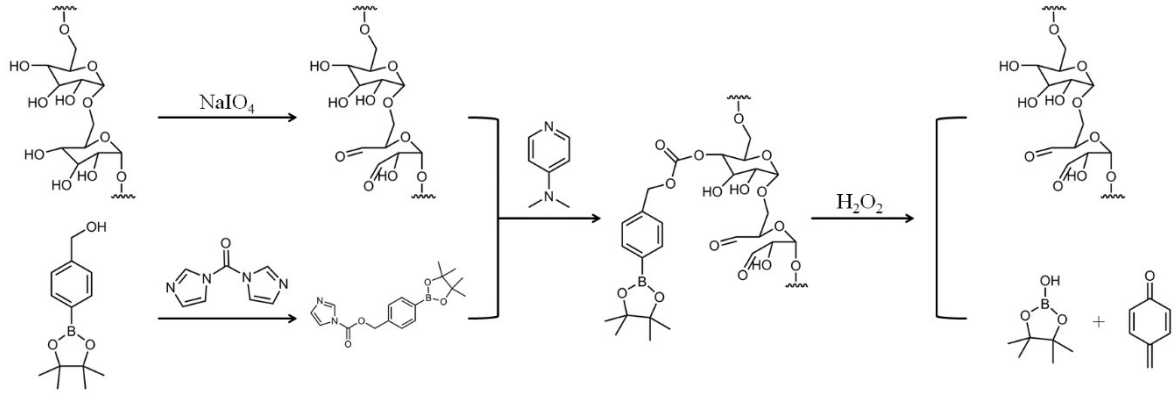

Fig. S1 Schematic illustration of the synthesized POD and its degradation product.

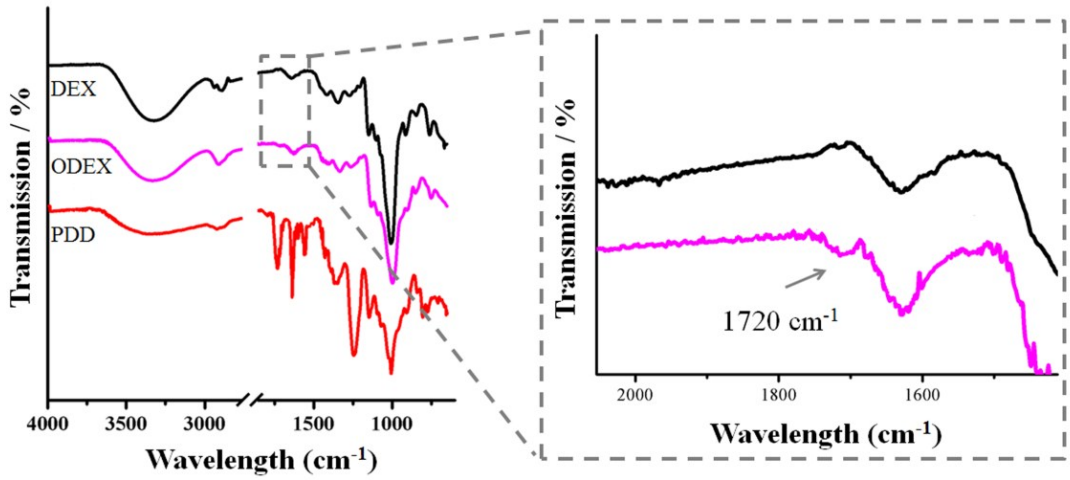

Fig. S2 FTIR spectra of DEX, ODEX and POD suggested the successful modification of each step.

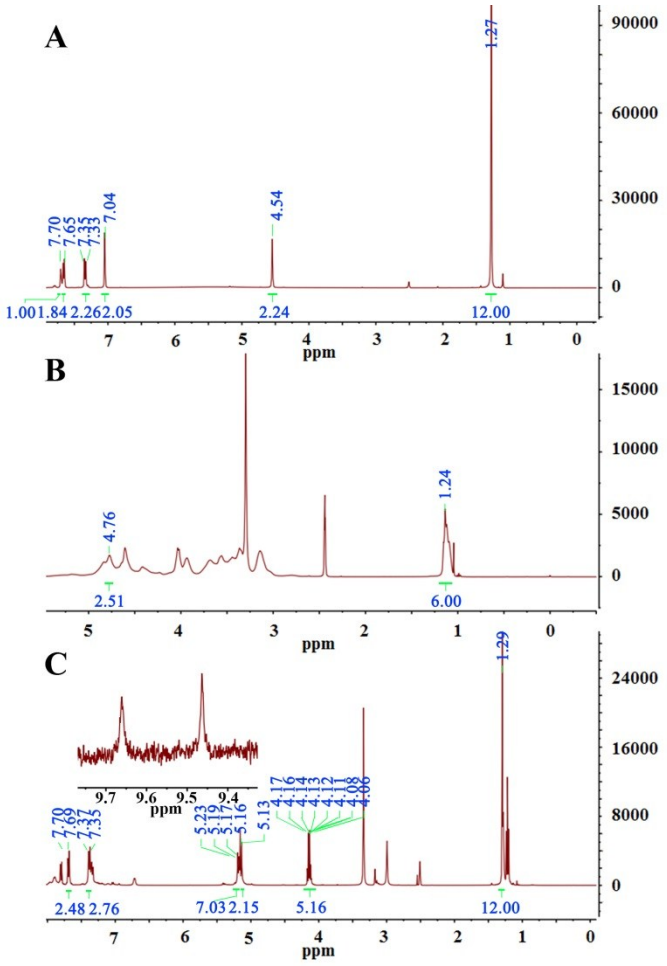

Fig. S3 (A) ^{1}H NMR data for PAPE-CDI, **(B)** ^{1}H NMR data after ODEX reacting with ethyl carbazate to detect the oxidation degree of ODEX, **(C)** ^{1}H NMR data for POD, the peaks showing at δ 9.46 and δ 9.67, suggesting the existence of aldehyde groups after modification.

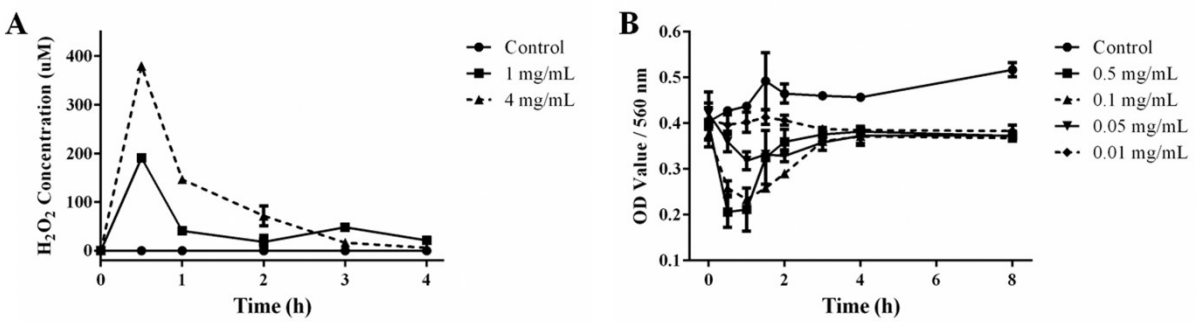

Fig. S4 (A) DCFH-DA assisted method detected GOx induced H_2O_2 generation at different glucose concentration (0, 1 and 4 mg mL^{-1}), **(B)** different concentrations of GOx induced gluconic acid generation within high glucose DMEM medium (4 mg mL^{-1}) lead to the same degree of pH drop.

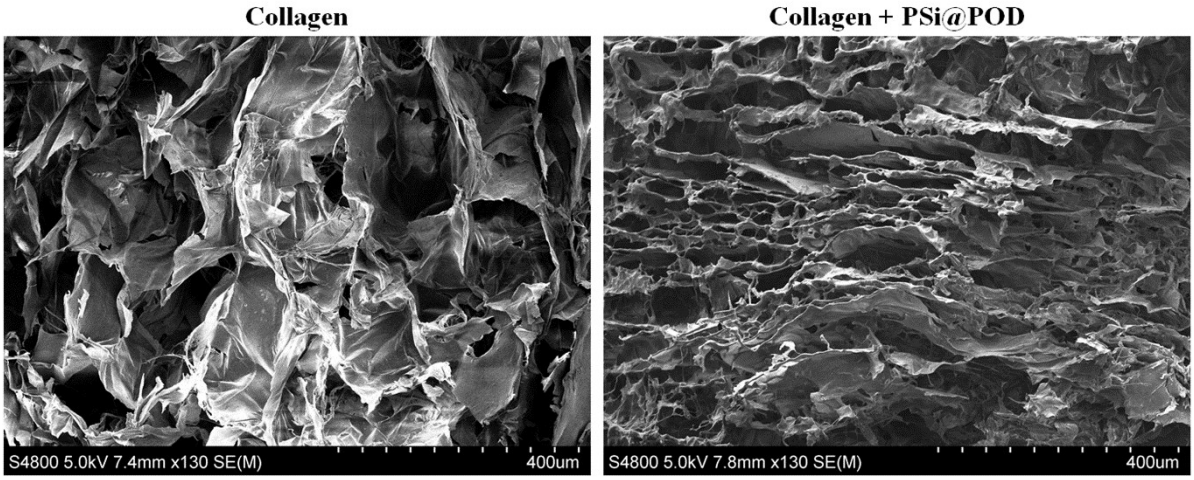

Fig. S5 SEM images of bare collagen or PSi@POD embedded collagen after lyophilization. Collagen was first fully dispersed in dd-H_2O at 40 °C, after cooling to room temperature, dd-H_2O or PSi@POD was added in the collagen solution followed by lyophilization.

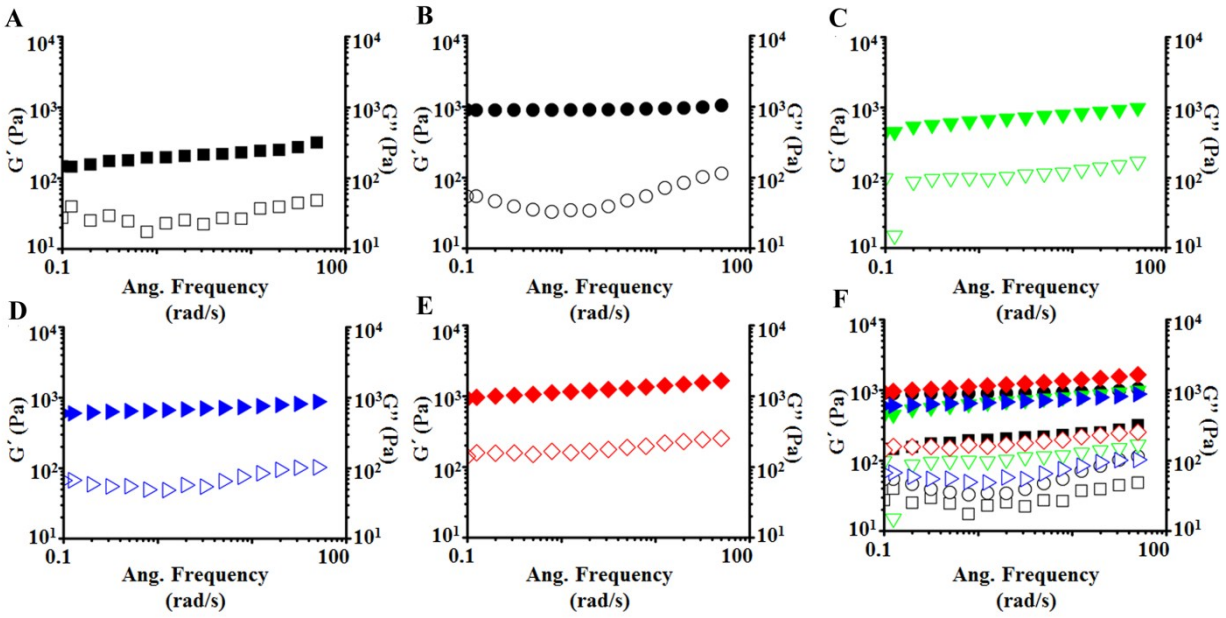

Fig. S6 Rheological dynamic angular frequency sweep tests of **(A)** C, **(B)** COD, **(C)** CP, **(D)** CP1, **(E)** CP4 and **(F)** the stacking figure. The solid data point in A–E and F correspond to the storage moduli G'. The open data point in A–E and F correspond to the loss moduli G". In F, black square, black circle, green down-triangle, blue right-triangle and red diamond, respectively, correspond to C, COD, CP, CP1 and CP4.

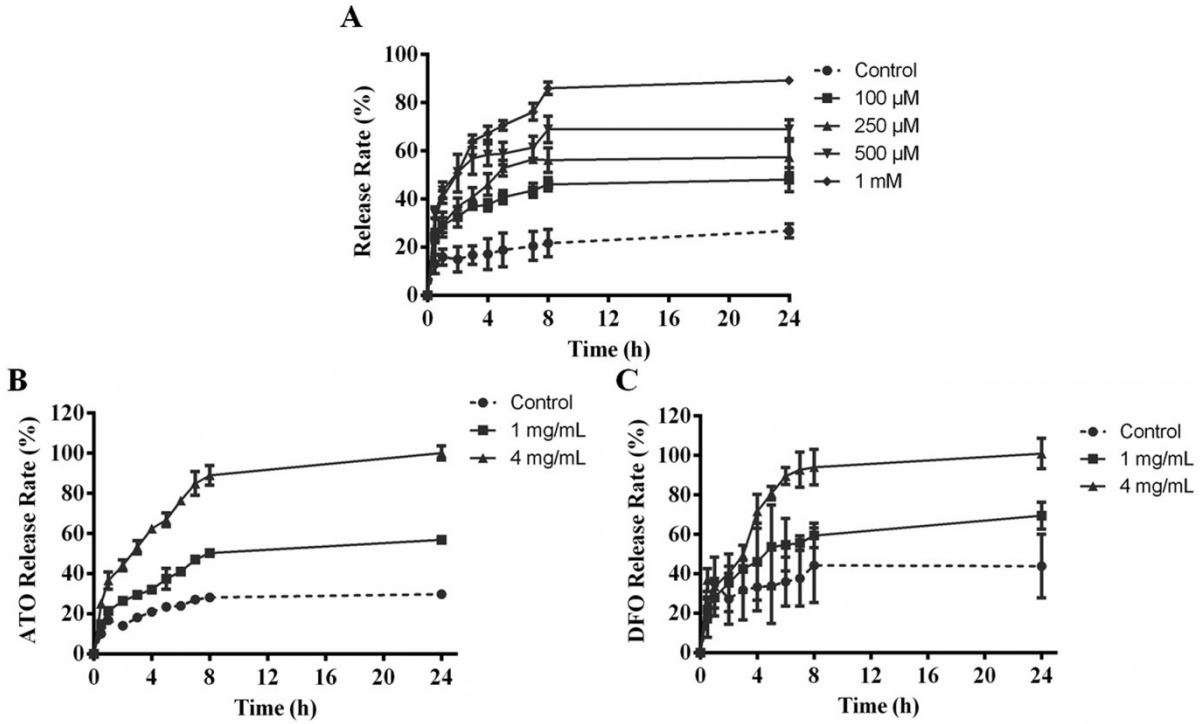

Fig. S7 (A) H_2O_2 concentration dependent ATO release from PSi@POD without adding GOx. **(B)** ATO and **(C)** DFO release behavior from D-G-PSi@POD within PBS buffer at different glucose concentration (0, 1 or 4 mg mL^{-1}) under stirring condition for 24 h. Data are shown as mean ± SD (n = 3).

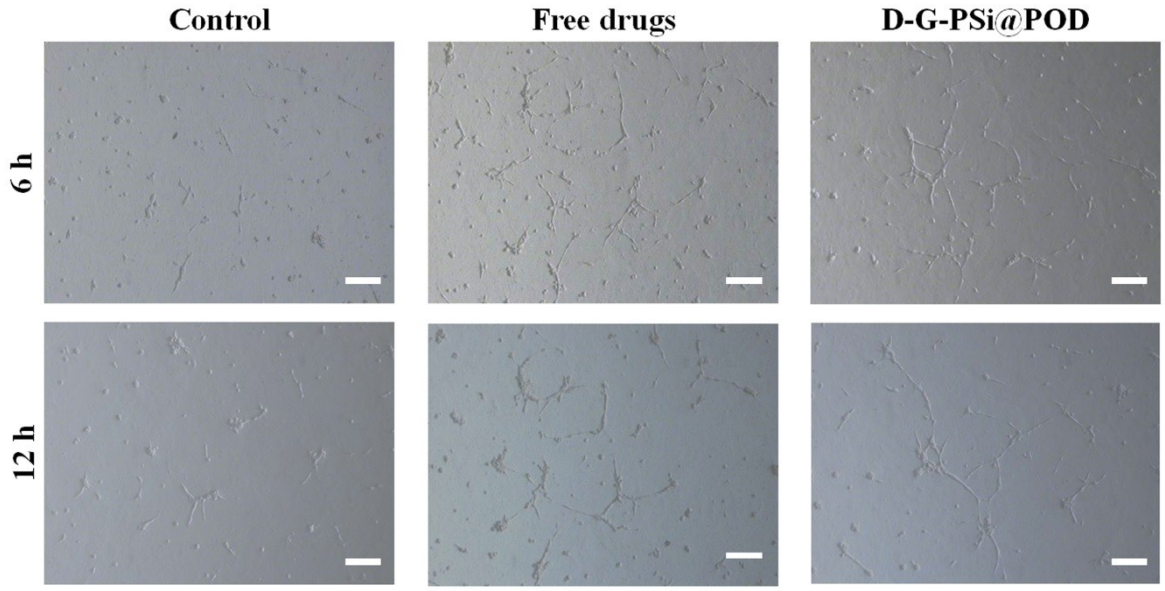

Fig. S8 Optical microscopic images of the tube formation assay on control, free drugs and D-G-PSi@POD groups at 6 and 12 h. All scale bars are 200 μm.

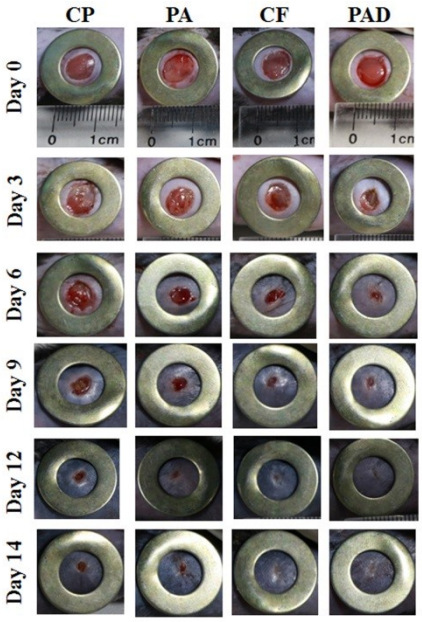

Fig. S9 Digital photographs of the wounds at day 0, 3, 6, 9, 12 and 14 after injuries.

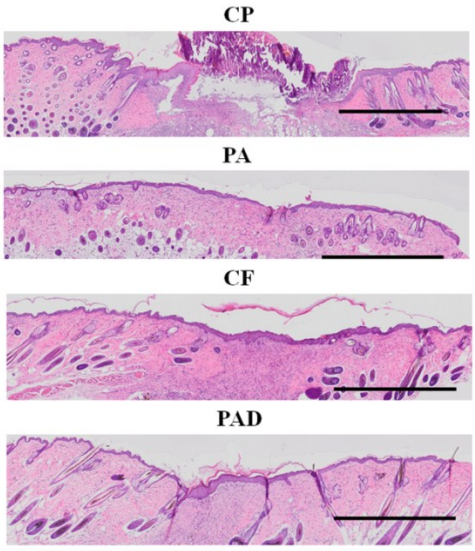

Fig. S10 H&E stained full-thickness wound bed of different groups on day 14. Except for CP group, all other three groups showed nearly full dermis coverage. Comparing to CF, PAD showed less regenerative granulation tissues, suggesting the detained regenerative efficacy from PAD group at late stage. All scale bars are 1 mm.

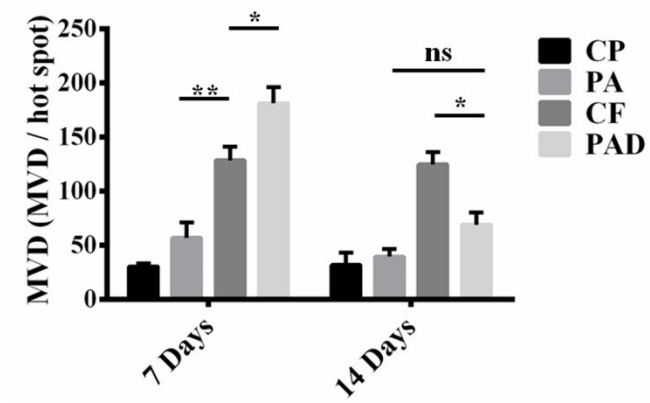

Fig. S11 Semi-quantitative evaluation of microvessel density (MVD) for each group on day 7 and day 14 post-operation. Data are shown as mean ± SD.

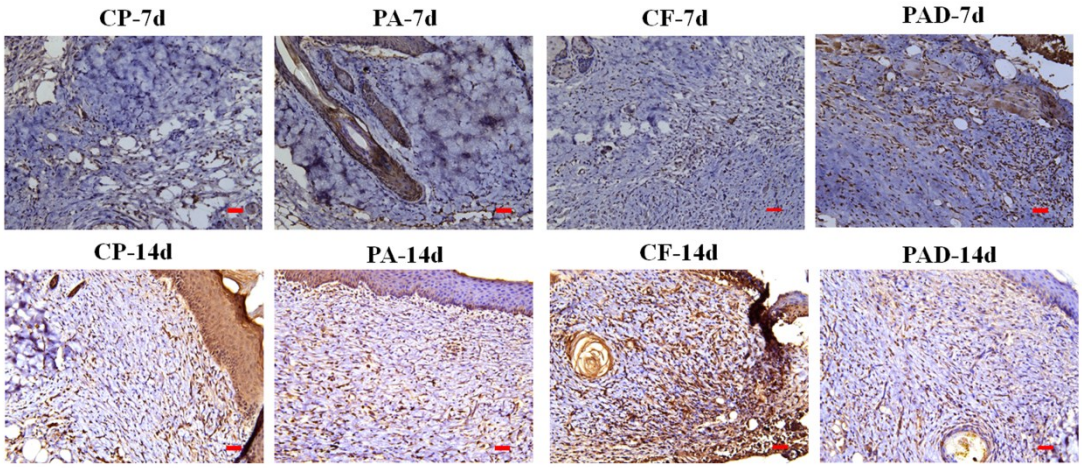

Fig. S12 Ki-67 immunohistochemical staining of the wound sites at 7 d or 14 d after treatment with different formulations. All scale bars are 200 μm.

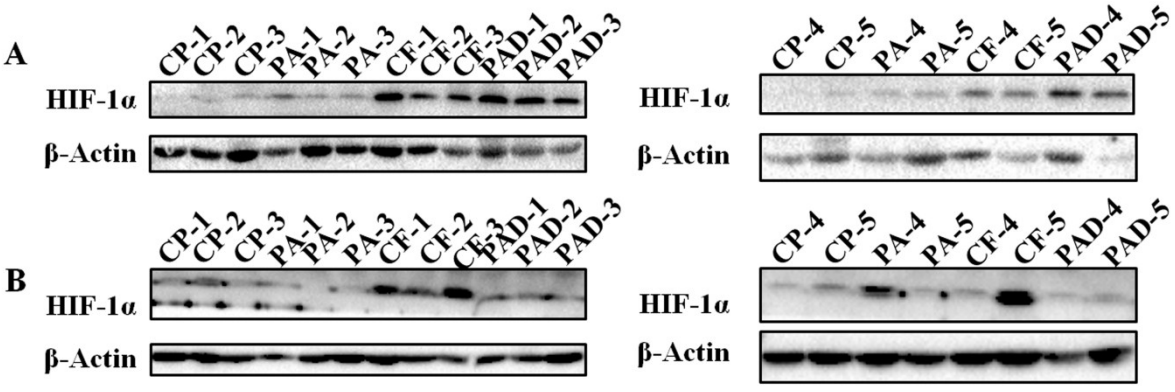

Fig. S13 Western blot analysis of each skin tissues at **(A)** day 7 or **(B)** day 14 for semi-quantitative protein analysis.

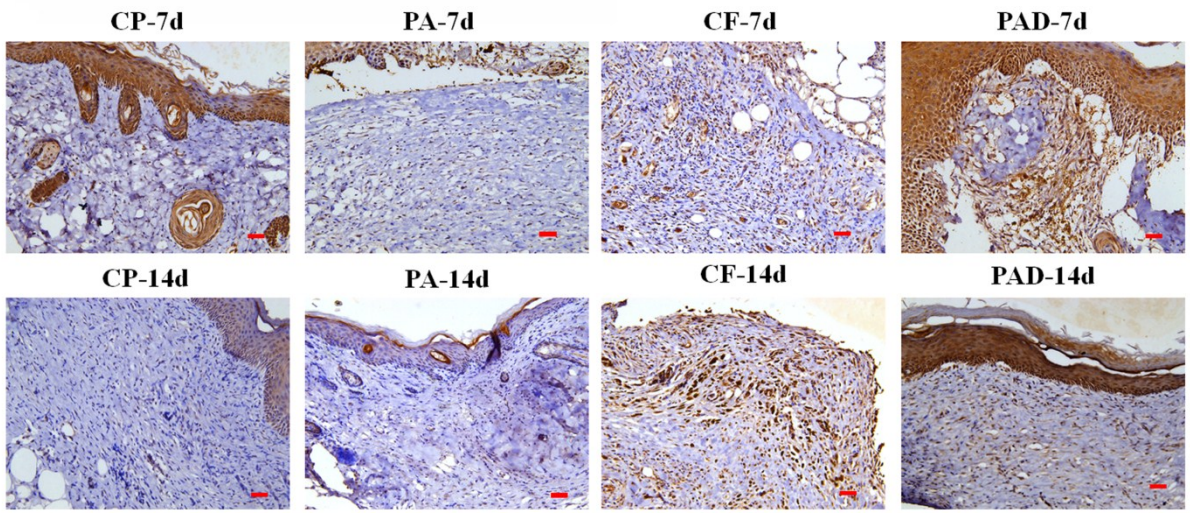

Fig. S14 Immunohistochemical staining of VEGF within the wound sites at 7 d or 14 d after treatment with different formulations. All scale bars are 200 μm.

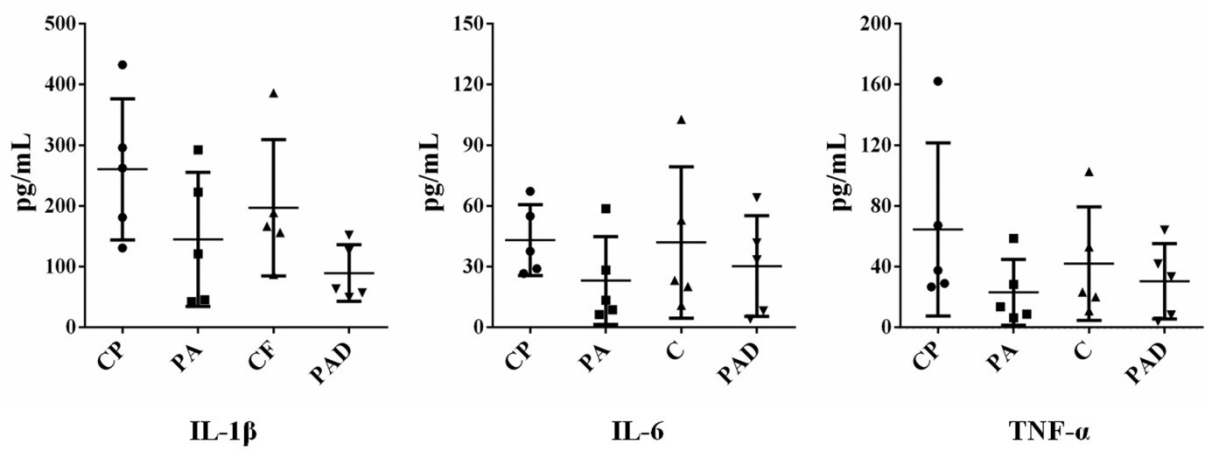

Fig. S15 Quantitative evaluation of major pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α) secretion within the wound site after 14 d treatment. Results are shown as mean ± SD, n = 5.

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