Supporting Information

for

Substrate-Independent Polymer Coating with Stimuli-Responsive Dexamethasone Release for On-demand Fibrosis Inhibition

Pingyun Yuan[1,2#], Xinyu Qiu[3#], Tao Liu[1], Ran Tian[1], Yongkang Bai[1], Shiyu Liu*[3], Xin Chen[1]* and Yan Jin[3]

[1] School of Chemical Engineering and Technology, Shaanxi Key Laboratory of Energy Chemical Process Intensification, Institute of Polymer Science in Chemical Engineering, Xi'an Jiao Tong University, Xi'an 710049, P. R. China

[2] Xi'an Jiao Tong University Shenzhen Research School, High-Tech Zone, Shenzhen, 518057, P. R. China

[3] State Key Laboratory of Military Stomatology & National Clinical Research Center for Oral Diseases & Shaanxi International Joint Research Center for Oral Diseases, Center for Tissue Engineering, School of Stomatology, Fourth Military Medical University, Xi'an, Shaanxi 710032, China

[#] These authors contributed equally to this work.

* Corresponding Author (E-mail: chenx2015@xjtu.edu.cn; liushiyu@vip.163.com)

1 Materials

APEG (Mn =500 Da) was purchased from Maya Reagent, China. Dexamethasone (Dex) was purchased from Shanghai Yuanye Co. Ltd. Succinic anhydride (SA), N-hydroxysuccinimide (NHS), dimethylaminopyridine (DMAP), dicyclohexylcarbodiimide(DCC), mercaptoethyl amine(MEA), N, N-Diisopropylethylamine (DIPEA) , 2, 2-dimethoxyl-2-phenylacetophenone (DMPA), PDDA (Mw 100000-200 000, 20 wt % in H_2O), PSS (Mw 70 000), fluorescein isothiocyanate (FITC) were purchased from Sigma-Aldrich. $CHCl_3$, Dimethyl Formamide (DMF), Pyridine, tetrahydrofuran (THF), isopropanol, ethanol, H_2SO_4 (98%), H_2O_2 (30%), NaCl were purchased from Sinopharm Chemical Reagent Co., Ltd (Xi'an, China). Polycaprolactone (PCL, MW 80000) was purchased from Shanghai Titan technology co., LTD. MilliQ water was prepared using a MilliQ system (Bedford, MA, America).

2 Synthesis of APEG-COOH

APEG (500 mg, 1 mmol) was dissolved in pyridine (10 mL). A solution of SA (0. 30 g, 3 mmol) and DMAP (12 mg, 0.1 mmol) in pyridine (4 mL) was added to the dexamethasone solution. The reaction was stirred overnight under nitrogen at room temperature. Pyridine was then removed under vacuum, and 16 mL water was added into the residue to dialyze (MW 100) against deionized water for 3 d before freeze dry to obtain the APEG-COOH.

3 Synthesis of DEX-SH

DEX-COOH: Dexamethasone (1.0 g, 2.55 mmol) was dissolved in pyridine (25 mL). A solution of SA (0.77 g, 7.65 mmol) and DMAP (31mg, 0.255 mmol) in pyridine (10 mL) was added to the dexamethasone solution. The reaction was stirred overnight under nitrogen at room temperature. Pyridine was then removed under vacuum and 40 mL water was added into the residue. Then mixture was stirred for 10 min and then centrifuged. The resulting precipitate was washed again with H_2O.

DEX-NHS: DEX-COOH (98.4 mg, 0.2 mmol) and NHS (23 mg, 0.2 mmol) were dissolved in $CHCl_3$ (10 mL). After complete dissolution at room temperature (RT), DCC (41.2 mg, 0.2 mmol) was added then the solution was stirred at RT overnight. After freeze drying, DEX-NHS will be harvested without further purification.

DEX-SH: DEX-NHS (149 mg, 0.37 mmol) were dissolved in 4 mL DMF, then MEA (216 mg, 0.37 mmol) and N, N-Diisopropylethylamine (DIPEA, 101 μL, 0.61 mmol) was added into the mixture and further stirred over night at RT to yield DEX-SH. Dex-SH was purified with HPLC using water-acetonitrile added with 0.1% TFA as the eluent (from 7:3 to 1:9).

4 Synthesis of DEX-PEG-COOH

DEX-SH (55.2mg, 0.1mmol) was dissolved in 10 ml ethyl alcohol, then APEG-COOH (50mg, 0.1mmol) and DMPA (25.6mg) were added to the mixture, followed by freeze-pump-thaw three times to eliminate dissolved oxygen. Then the mixture was stirred at room temperature under long wavelength ultraviolet light (365 nm), provided by a UV light supplier to initiate the thiol-ene reactions. After 30 min, the light was turned off and the solution was further stirred overnight. The solution was dialyzed against ethyl alcohol and deionized water for 3 d before freeze dry to obtain the DEX-PEG-COOH.

5 Fabrication of PCL scaffold

For preparation of PCL scaffold, the sugar cubes were soaked in THF solution of PCL (10%) for 24h. After that, the composite was frozen with liquid nitrogen for 5min and freezing dry for 12h. The salt spheres were leached out in distilled water and freeze-dried again to obtain porous scaffolds.

6 Preparation of $(PDDA/PSS)_9$ films on different substrates

Prior to use quartz slides were cleaned *via* immersion in piranha solution (1:3 (v/v) mixture of 30% H_2O_2 and 98% H_2SO_4), followed by heating until no bubbles were released. PCL scaffolds were cleaned by consecutive sonication in isopropanol and ethanol, the sonication time was 10 min for each cleaning step. $(PDDA/PSS)_9$ films were prepared on cleaned substrates (teflon slides or PCL scaffold) by alternating immersion of the substrates into the aqueous solutions of PDDA(1 mg/mL) and PSS (1 mg/mL) containing NaCl (1.0 M) for 20 min until the desired layer number was reached. Each immersion step was followed by thorough rinsing with water. After the desired layer number was reached, the resulting film were thoroughly rinsed by water and gently dried with N_2 flow.

7 Fabrication of DEX-PEG-COOH loaded $(PDDA/PSS)_9$ film (DEX-PEG-($PDDA/PSS)_9$)

(PDDA/PSS)9 coated quartz and PCL scaffolds were immersed into the ethyl alcohol solution of DEX-PEG-COOH (pH=7.2) at room temperature for 4min, followed by thorough rinsing with ethyl alcohol and drying with N_2 flow.

8 Western blots

The skin tissue was harvested in a RIPA lysis buffer which contain protease inhibitor (Beyotime, China). After whole protein extracted, they were quantified by BCA assay (Beyotime, China) and separated on 6%-15% polyacrylamide gels. Then, they were transferred to PVDF membranes (Millipore, USA), blocked in 5% BSA in TBST, and hybridized with antibodies GAPDH (Cell signaling, USA), Collagen I (Abcam, USA), TGF-β (Abcam, USA) and Fibronectin (Abcam, USA), respectively. Finally, signals were revealed after incubation with secondary antibodies at room temperature (Cwbiobiotech, China) coupled to peroxidase by using ECL (Tanon, China).

9 Immunofluorescence

The sections from skin tissue were fixed and rinsed. After rinsing, they were permeabilized with 0.03% Triton-X100 for 15 min at room temperature and blocked in 5% BSA for 1 hour. Then, the sections were incubated overnight (at least 12 hours) at 4 ℃ with primary antibody of α-SMA (Abcam, USA). After rinsing by PBS, the sections were incubated with fluorescence secondary antibody (Cell signaling, USA) at room for 1 hour. Finally, the nuclei were counterstained by Hoechst 33342 (Sigma-Aldrich, USA) for 15 minutes at room temperature. The results were examined under the confocal microscope (Olympus, Japan). The photographs were evaluated by Image Pro Plus software (Media Cybernetics, USA) from three randomly selected views of each specimen.

10 Histological analysis

The harvested tissue sections from skin were fixed in 4% phosphate-buffered

paraformaldehyde for 48 hours, embedded in paraffin. Serial sections of 4μm-thick were cut from the blocks and underwent H&E staining as well as Masson's Trichrome staining following the manufacturer's instructions (BASO, China). Photographs were captured by stereoscopic microscope (Leica, Germany) and percentage of collagen fibers in total area was measured by Image Pro Plus software (Media Cybernetics, USA). In addition, the statistical analysis of the number of hair follicles was also obtained by the H&E stained micrographs.

11 Construction of cutaneous wound healing model

All animals were purchased from the Animal Center of Fourth Military Medical University, Xi'an, China. The animal protocols were approved by the Animal Ethical and Care Committee of the Fourth Military Medical University (Approved ID: No. 19113). All animals were housed under specific pathogen-free conditions (24 °C, 12-hour light/12-hour dark cycles) with free access to food pellets and tap water as required. Twelve-week-old female C57BL/6J mice were randomized to three groups, each mouse was anesthetized intraperitoneally with 1% sodium pentobarbital, and the skin was prepared. Then, the punch biopsy instrument (1 cm diameter) was placed with moderate force onto the dorsum of the mouse to create an impression of the circumference to make the full-thickness wound on the mid-back. Next, the middle of the outline region of skin was sharply excised along the outline with a pair of scissors. The excised tissue was full-thickness skin in depth, leaving the subcutaneous dorsal muscle exposed after excision. The scaffolds were then placed onto the wound area and covered by two layers of Vaseline gauze with discontinuous suture onto the marginal recipient skin of the defect area by 5-0 silk suture for three days.

12 Statistical analysis

Data were expressed as mean ± SD as indicated. Comparisons between multiple group comparisons were performed by one-way ANOVA. Bonferroni correction was applied when multiple comparisons were performed. *P* values less than 0.05 were considered statistically significant. Graphs and statistical analysis were performed by using GraphPad Prism (GraphPad Software, USA) and SPSS software (IBM, USA).

13 Characterization

The overall morphology of the coating was examined using scanning electron microscopy (SEM) (Hitachi S-4800 with energy dispersive spectrometer) equipped with energy dispersive spectrometer (EDS) after gold coating of the microsphere samples on a stub. The infrared (IR) spectra were measured by AVATAR 320 FT-IR using KBr pellets. All pH value measurements were carried out on a Sartorius BECKMAN F 34 pH meter. The nuclear magnetic spectra of COOH-DEX-PEG was measured by nuclear magnetic resonance spectrometer (NMR) (AVANCE III HD 600 MHz). The FITC-DEX release was monitored by fluorescence microscopy using an Olympus BX51 microscope equipped with a fluorescent lamp; ex = 495 nm, em = 525 nm.

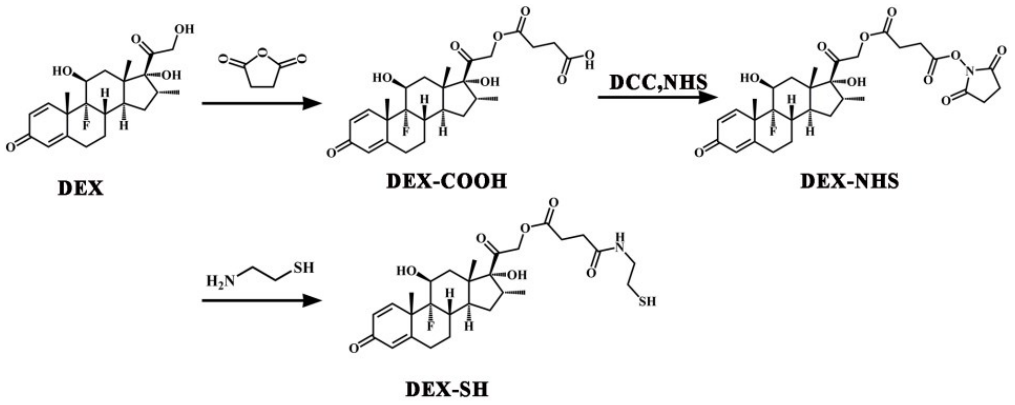

Figure S1. Schematic illustration of synthesis of DEX-SH.

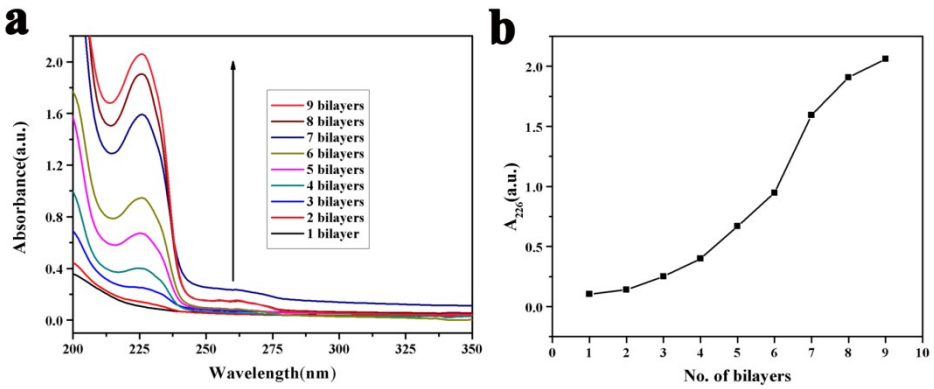

Figure S2 a) UV–vis spectrometry of the (PDDA/PSS)n film with different number of bilayers. b) the stepwise increase of the UV–vis absorbance at 226 nm arising from the phenyl groups of PSS against the growth of PDDA/PSS bilayers.