Electronic Supplementary Information

Functional electrospun fibrous scaffolds with dextran-*g*-poly(L-Lysine)-VAPG/microRNA-145 to specially modulate vascular SMCs

Peiqiong Zhou, Fang Zhou, Bo Liu, Yunhui Zhao, Xiaoyan Yuan[*]

School of Materials Science and Engineering, and Tianjin Key Laboratory of Composite and Functional Materials, Tianjin University, Tianjin 300350, China

[*]E-mail: yuanxy@tju.edu.cn; xyuan28@yahoo.com.

Materials

Dextran (Dex, molecular weight ~6000) was purchased from Sigma-Aldrich Trading Co., Ltd., (Shanghai, China). Poly(L-lactide-*co*-glycolide) (PLGA, LA/GA=75/25, $\overline{M}_n$=7.5×10^4) and poly(ethylene glycol)-*b*-poly(L-lactide-co-ε-caprolactone) (PELCL, LA/CL=75/25, $\overline{M}_n$=7.0×10^5) were synthesized in our laboratory.[1] *N,N*'-carbonyldiimidazole (CDI), tetrahydrofuran (THF), ethylenediamine (ED), dimethyl sulfoxide (DMSO) and other solvents were provided by Tianjin Yuanli Chemical Reagent Co. Ltd., China. Trifluoroacetic acid (TFA) and hydrobromide (HBr) in acetic acid (33%w/w) were supplied by Aladdin biochemical Polytron Technologies Co. Ltd., China. Glycidyl methacrylate (GMA), 4-dimethylaminopyridine (DMAP), 2,2-dimethoxy-2-phenylacetophenone (DMPA) and *N*-ε-benzyloxycarbonyl-L-lysine were obtained from Bailingwei Technology Co., Ltd., China. MicroRNA-145 (miR-145) mimic (sense 5'-GUC CAG UUU UCC CAG GAA UCC CU-3', antisense 5'- GGA UUC CUG GGA AAA CUG GAC UU-3'), negative control miRNA (NC), carboxyfluorescein-labeled miRNA (FAM-miRNA) and VAPG-SH peptide sequence (Val-Gly-Val-Ala-Pro-Gly-Cys) were supplied by Gene Pharma Co., Ltd., China. Cell counting kit-8 assay (CCK-8) was purchased from Dojindo, Japan. Heparin sodium (159 units/mg) was obtained from Shanghai Chemical Reagent, China. Phosphate buffer saline (PBS) solution (pH 7.4, 0.1 M), 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) and tris-acetate-EDTA (TAE) buffer were provided by Tianjin Runtai Technology Co., Ltd., China.

Synthesis of amino dextran (Dex-NH_2)

Hydroxyl groups of dextran (Dex) were firstly activated with CDI according to the reference.[2] In brief, a solution of CDI (30 molar equivalent to dextran) was added dropwise to the dextran solution in anhydrous DMSO. The reaction was carried out at room temperature under the nitrogen atmosphere for 24 h. Then, excessive ED (2.5 mL) in 6 mL anhydrous DMSO was added dropwise to the resultant mixture, and the reaction was kept for additional 48 h. After that, excess acetone was adapted to precipitate the reaction mixture to obtain the raw Dex-NH_2. Then, the raw Dex-NH_2 was dissolved in a small amount of deionized water

and dialyzed (MWCO 2 kDa) against DI for 48 h at room temperature followed by lyophilization.

Synthesis of dextran-*g*-poly(*N*-ε-benzyloxycarbonyl-L-lysine) (Dex-*g*-Plys(Z))

Dex-*g*-Plys(Z) was prepared by the ring opening polymerization of L-lysine NCA, which was initiated by Dex-NH_2. Firstly, 1 g lysine NCA and predetermined amount of initiator (Dex-NH_2) were dissolved in 4 mL anhydrous DMSO respectively, where the molar feed ratios were 1/15, 1/30, 1/50. The reactions were performed under a dry nitrogen atmosphere with vigorously stirring at 50°C for 72 h. Then, Dex-*g*-Plys(Z) was precipitated with excessive diethyl ether and the precipitate was washed with diethyl ether trice before lyophilization.

Deprotection of Dex-*g*-Plys(Z)

Dex-*g*-Plys(Z) (1.00 g) was dissolved in 10 mL trifluoroacetic acid, and followed by adding 1.67 mL HBr in acetic acid (33% w/w) into the above solution. The reaction mixture was kept in ice bath for 30 min and then stirred vigorously for 48 h at room temperature for the complete deprotection. Afterwards, acetone was used to precipitate the Dex-*g*-poly(L-lysine) (Dex-*g*-PLL) from the corresponding reaction mixture. Then the precipitate was re-dissolved with the least amount of dionized water and acetone was added again to purify the crude product. After that, the aqueous solution was neutralized with saturated sodium bicarbonate solution. Dex-*g*-PLL was dialyzed against DI for 72 h at room temperature followed by lyophilization.

Synthesis of dextran-*g*-poly(L-lysine)-VAPG (Dex-*g*-PLL-VAPG)

Dex-*g*-PLL (20mg) and GMA (60 μL) was dissolved in 2 mL mixed solvent of methanol and dionized water (10:1, *v*/*v*). The reaction was kept at 15°C for 20 h with gently stirring. Dex-*g*-PLL-GMA was obtained by precipitating the above mixture with diethyl ether followed by vacuum drying. Afterwards, Dex-*g*-PLL-GMA and VAPG-SH peptide was dissolved in the above mixed solvent methanol and dionized water in a petri dish. A small amount of DMPA was added into the above mixture with gently stirring for the complete dissolution. The solution was irradiated under the ultraviolet light (365 nm) for 20 min with DMPA as a photoinitiator. Dex-*g*-PLL-VAPG was attained by precipitation in ethanol, then dissolved in

dionized water, and precipitated in ethanol. The procedure was repeated thrice to remove the unreacted residues. Finally, the crude product was dialyzed against deionized water (MWCO 7 kDa) and collected until lyophilization was completed.

^{1}H NMR analysis

^{1}H NMR spectra of polymers were measured on a Bruker ARX 400MHz spectrometer using D_2O, $CDCl_3$ or DMSO-d_6 as the solvents at room temperature. Herein, some hydroxyl groups of dextran were firstly activated by CDI and modified with ED to produce the initiation sites as reported previously.[2] As shown in Figure S1, the characteristic signal of anomeric carbon (a, O-CH-O) at δ 4.92 ppm was found. The peaks at δ from 3.38 to 4.02 ppm corresponded to the methylidyne and methylene protons (b, CH-O and CH_2-O) of dextran. The peaks at δ 2.67 and 3.16 ppm belonged to methylene protons linked to amide (c, $\underline{CH_2}$-NH-CO) and amino groups (d, $\underline{CH_2}$-NH_2), respectively. The degree of substitution of amine group (defined as the percentage of primary amine groups linked to the glucose units of dextran) was calculated to be 45% according to the area ratio of peak b and d. Subsequently, Dex-*g*-PLL polymers with different molecular weights were prepared via ring opening polymerization of L-lysine *N*-carboxyanhydride (L-lysine NCA) by using Dex-NH_2 as the macroinitiator and then deprotected in HBr in acetic acid (33% w/w) with trifluoroacetic acid as the solvent. The peak at 4.21 ppm and 2.91 showed the methylidyne protons of CO-$\underline{CH}(CH_2)$-NH (a) and methylene protons of CH-$\underline{CH_2}$-CO (e), respectively. The peaks at δ from 1.2 to 1.8 ppm represented the continuous methylene protons in the chain of PLL. In this work, the amount of PLL side chains coupled to dextran bones were controlled by the molar feed ratios of [amino group]:[lysine NCA], designed as 1/15, 1/30 and 1/50. With the increasing of molar feed ratio, the molecular weights of Dex-*g*-PLL increased from 2.10×10^4 to 6.56×10^4. In order to endow the polycations with active targeting for VSMCs, VAPG-SH peptides were linked onto the Dex-g-PLL chains. Firstly, GMA was introduced to graft double bonds onto the chains, where the epoxy groups in GMA was employed to react with partial amino group in the chains. The specific signal of double bond appeared in ^{1}H NMR spectrum proved the successful synthesis. Then, VAPG-SH peptide was grafted onto Dex-*g*-PLL chains by thiol-ene addition reaction owing to the mercapto group (-SH). Herein, the disappearance of

specific shift of double bonds at 5.7 ppm and 6.1 ppm and the existence of some peaks such as 0.9~1.2 ppm, 3~4 ppm and 8 ppm nearby concomitantly confirmed the perfect grafting of VAPG peptide.

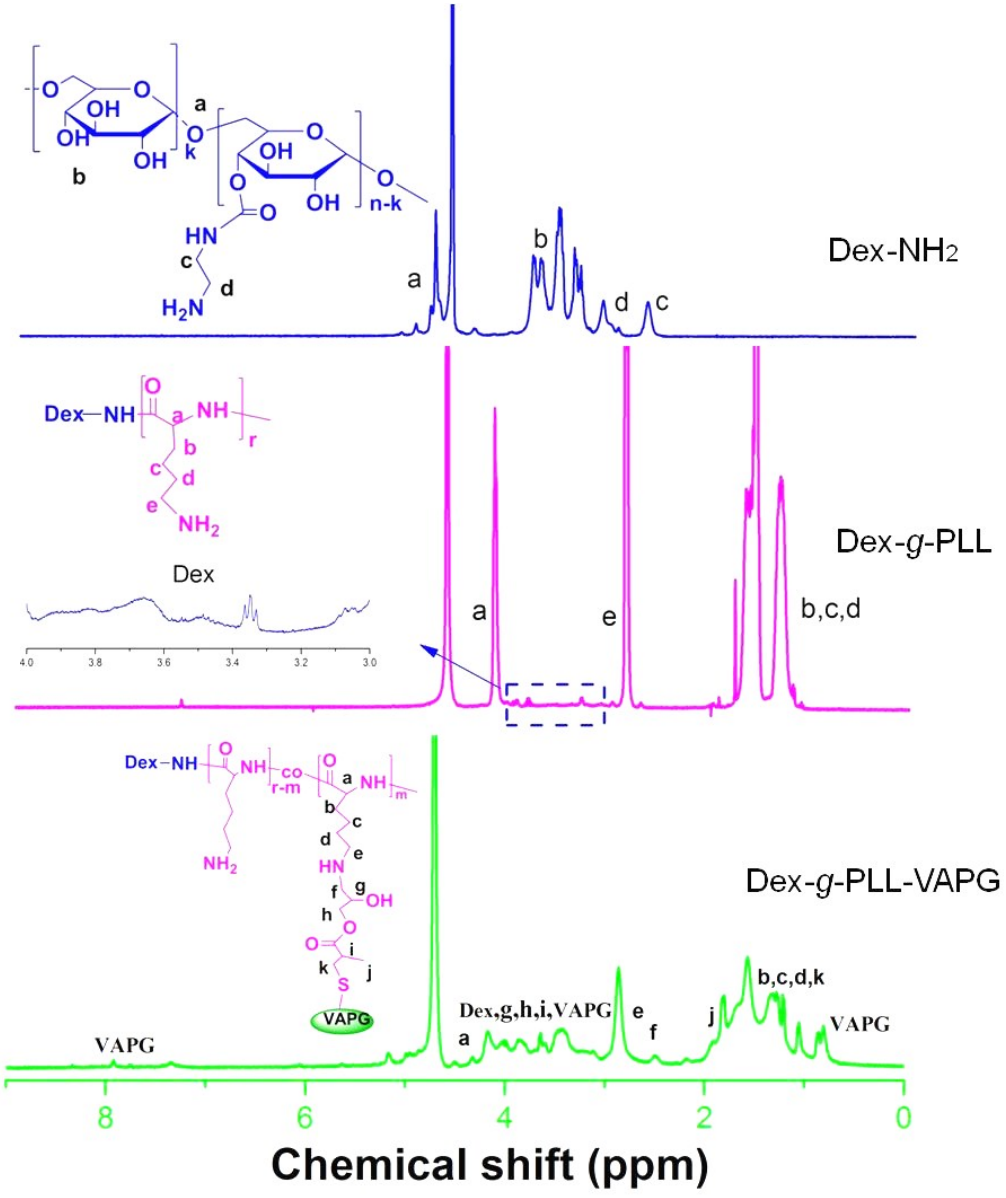

Figure S1. ^{1}H NMR spectra of Dex-NH_2, Dex-*g*-PLL and Dex-*g*-PLL-VAPG

Serum stability of the polymer/microRNA complexes

The serum stability of polymer/miRNA complexes was evaluated by gel electrophoresis using naked miRNA as control. Briefly, polymer/miRNA complexes and naked miRNA were added in PBS containing 50% FBS at 37°C with gently oscillation. Then, at each predetermined time point (0, 12, 24, 48, 72 and 96 h), 5 μL of each sample was extracted and kept at -20°C prior to gel electrophoresis measurement. As shown in Figure S2, the naked miRNA started to degrade after 24 h while complete degradation did not happen for the miRNA complex even after 96 h. It was suggested that Dex-*g*-PLL-1 and Dex-*g*-PLL-VAPG could prevent miRNA from degradation effectively.

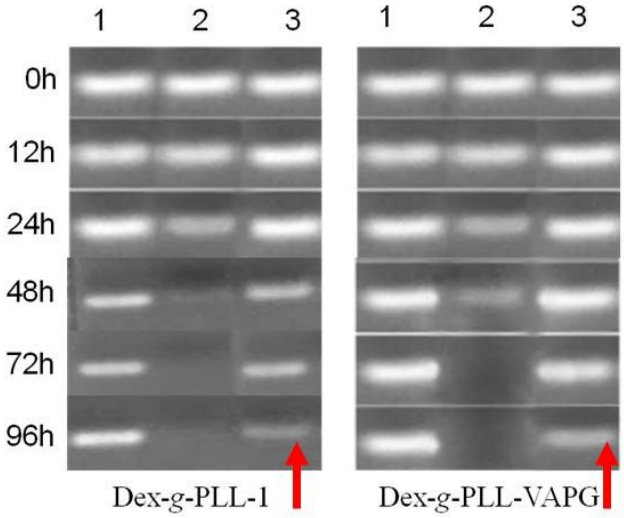

Figure S2. MiRNA-145 serum stability. Lane 1: intact miRNA; Lane 2: naked miRNA; Lane 3 : Dex-g-PLL-1/NC and Dex-g-PLL-1-VAPG/NC with mass ratio of 2 and 4, respectively.

Morphology of electrospun membranes

The morphology of electrospun membranes were viewed under a scanning electron microscope (SEM, Hitachi SU1510, Japan) and the mean fiber diameters were calculated based on SEM micrographs. As shown in Figure S3(a~e), all the electrospun membranes exhibited uniform morphologies absent of beads. LCSM was also employed to confirm the existence of miRNA complexes in the electrospun fibers by loading with carboxyfluorescein-labeled miRNA (FAM-miRNA). Figure S3(f,g) demonstrated homogeneous distributions of green fluorescence in samples PLGA/Dex-*g*-PLL containing FAM-miRNA (PLGA/DP(FAM-miRNA)) and PLGA/Dex-*g*-PLL-VAPG containing FAM-miRNA (PLGA/DPV (FAM-miRNA)).

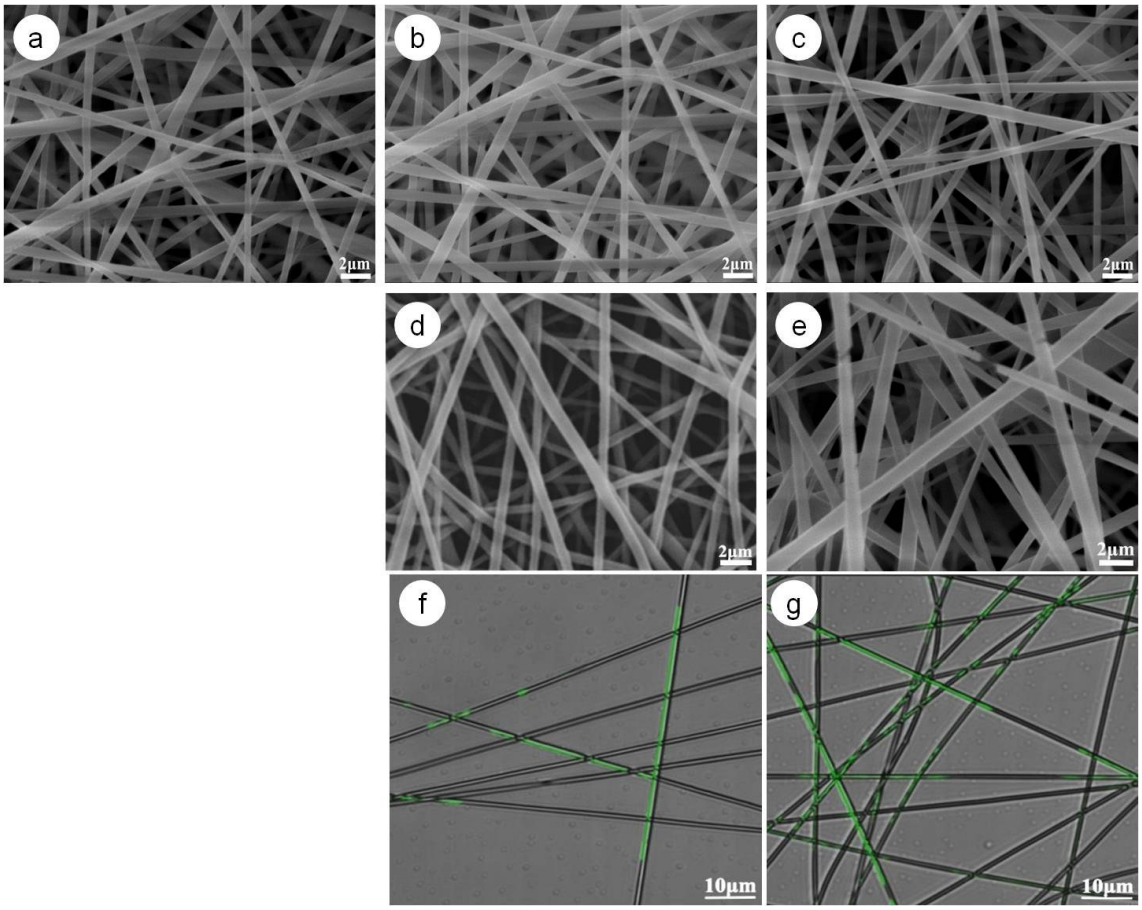

Figure S3. SEM images of PLGA (a), PLGA/DPnc (b), PLGA/DPVnc (c), PLGA/DPm (d) and PLGA/DPVm (e) electrospun membranes; LCSM images of PLGA/DP(FAM-miRNA) (a) and PLGA/DPV(FAM-miRNA) (b). Negative control miRNA (NC) and FAM-miRNA were employed.

SMC proliferation on the electrospun membranes

As shown in Figure S4, with the culture time increasing from day 3 to day 7, the SMCs distributed more and more densely and evenly on the electrospun membranes, representing the proliferation of SMCs. The amount of α-SMA on SMCs was trivial and the α-SMA exhibited random orientation on all the electrospun membranes after cell culture for 3 days. As the culture time increased, the α-SMA expression increased obviously. However, the morphology of SMCs and the quantity of α-SMA expression on SMCs were distinct for various samples. We found that the expression levels of α-SMA on the PLGA, PLGA/DPnc and PLGA/DPVnc electrospun membranes were similar to each other. And, the highest expression on the PLGA/DPm and PLGA/DPVm samples was also observed.

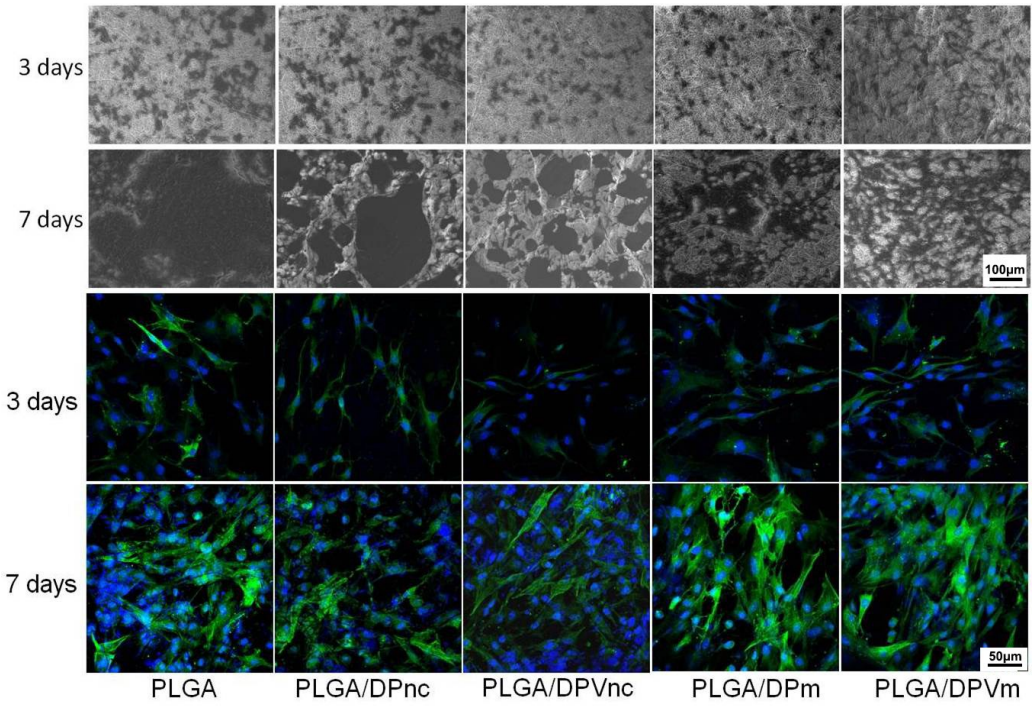

Figure S4. SMC proliferation, morphology and phenotype validation on the electrospun membranes containing miR-145 complexes (PLGA/DPm, PLGA/DPVm) in comparison to the pure PLGA electrospun membrane (PLGA) and the PLGA membranes containing the negative control miRNA complexes (PLGA/DPnc PLGA/DPVnc). SEM micrographs of SMCs spreading on the different electrospun membranes on day 3 and 7; LCSM micrographs of immunofluorescence staining of cultured SMCs with anti-α-SMA antibody on day 3 and 7 (Green, IgG Alex Fluor-488 labeled α-SMA; Blue, DAPI stained nuclei).

Reference

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2 H. Q. Song, X. B. Dou, R. Q. Li, B. R. Yu, N. N. Zhao and F. J. Xu, *Acta Biomater.*, 2015, **12**, 156-165.