

SUPPORTING INFORMATION

Aligned Carbon Nanotubes Reduce Hypertrophic Scar *via* Regulating Cell Behaviour

Weizong Weng^{†,⊥}, Sisi He^{‡,⊥}, Hongyuan Song^{§,⊥}, Xiaoqun Li^{//}, Liehu Cao[†], Yajie Hu[‡], Jin Cui[†], Qirong Zhou[†], Huisheng Peng^{‡,*} and Jiacan Su^{†,*}

[†] Department of Orthopaedics Trauma, Changhai Hospital, Second Military Medical University, Shanghai 200433, China

[‡] State Key Laboratory of Molecular Engineering of Polymers, Department of Macromolecular Science and Laboratory of Advanced Materials, Fudan University, Shanghai, 200438, China.

[§] Department of Ophthalmology, Changhai Hospital, Second Military Medical University, Shanghai 200433, China

^{//} Graduate Management Unit, Shanghai Changhai Hospital, Second Military Medical University, Shanghai 200433, China

[⊥] W.W., S.H. and H.S. contributed equally to this work.

* Corresponding authors:

Prof. Jiacan Su

Email: drsujiacan@163.com,

Prof. Huisheng Peng

Email: penghs@fudan.edu.cn

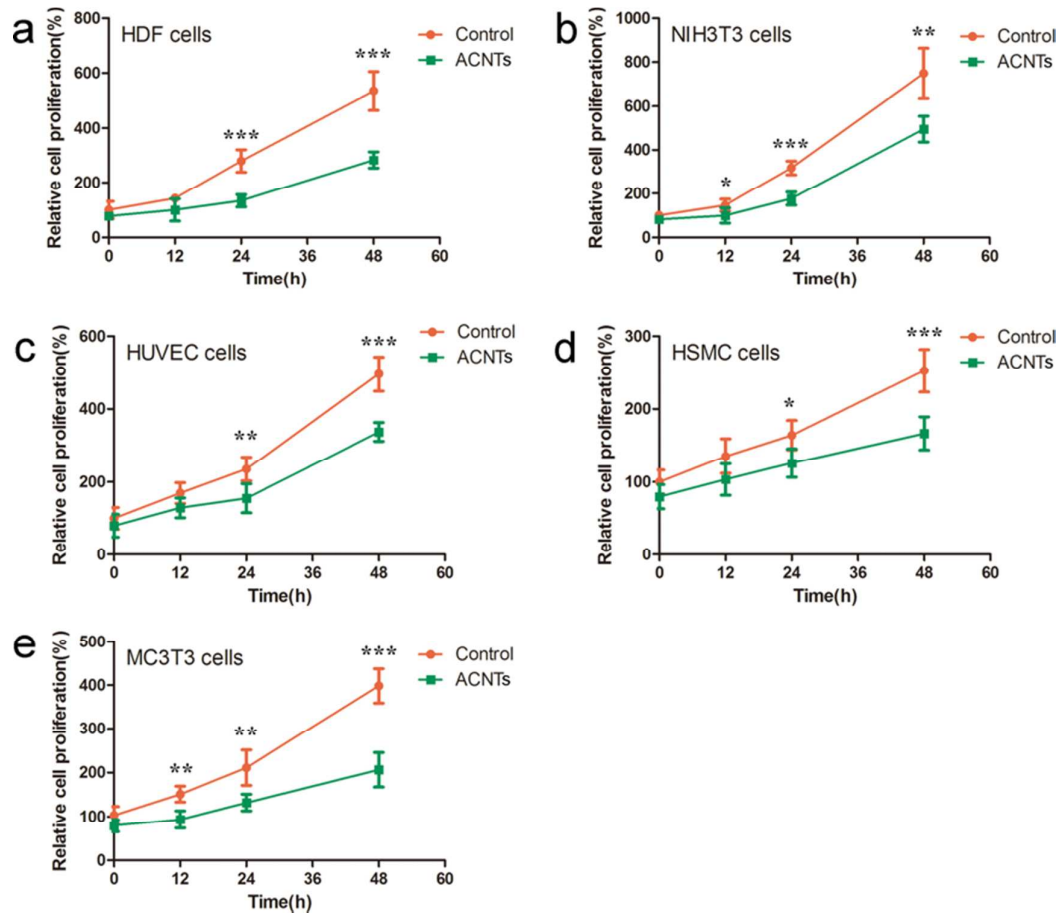


Figure S1. Multiple cells growth curve treated with ACNTs. (a) HDF cells, (b) NIH3T3 cells, (c) HUVECD cells, (d) HSMC cells, and (e) MC3T3 cells treated with ACNTs showed apparently inhibited cell growth compared with control group. The proliferation of cells was inhibited but not stopped as the cells growth curve continue to increase, which indicated that ACNTs might had no toxicity to cells. ($n=3$, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$).

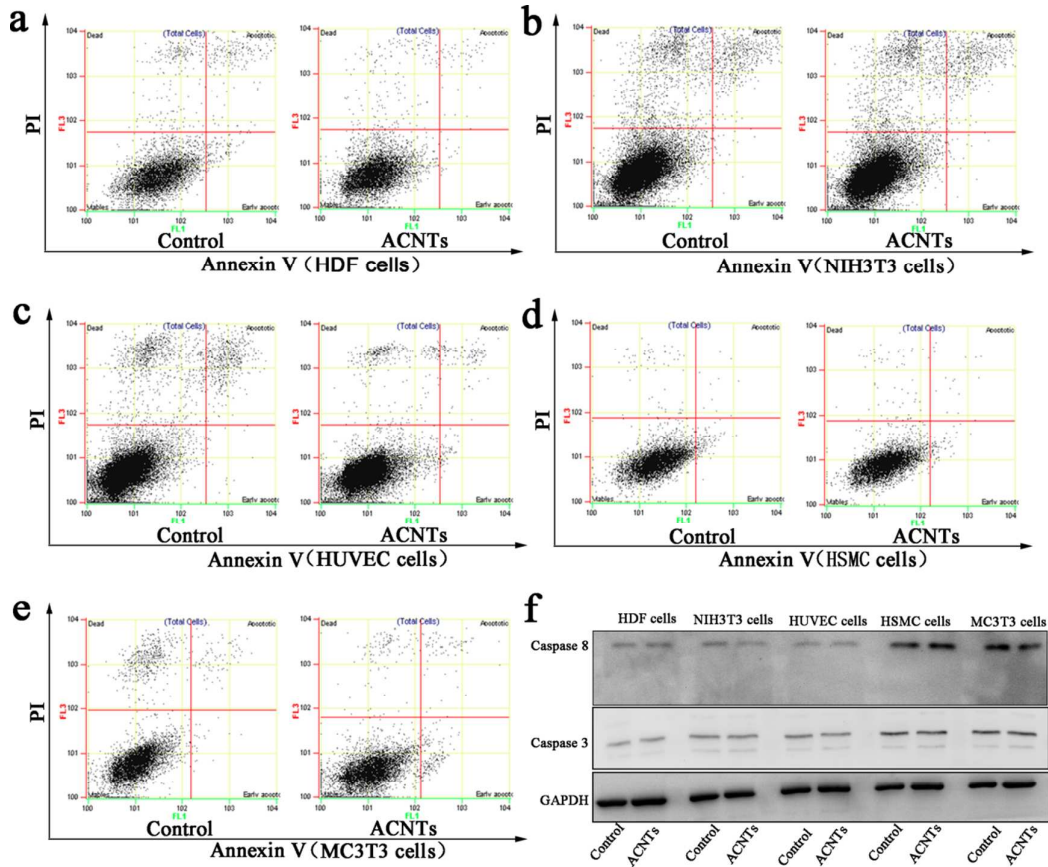


Figure S2. ACNTs did not induce cell apoptosis and necrosis. The apoptosis and necrosis of (a) HDF cells, (b) NIH3T3 cells, (c) HUVEC cells, (d) HSMC cells, and (e) MC3T3 cells were evaluated with flow cytometry, and the data showed that there were no significant different between ACNTs group and control group. (f) The cleavage of apoptosis related proteins, caspase 3 and caspase 8, were assessed using western blot, which showed no apparent difference.

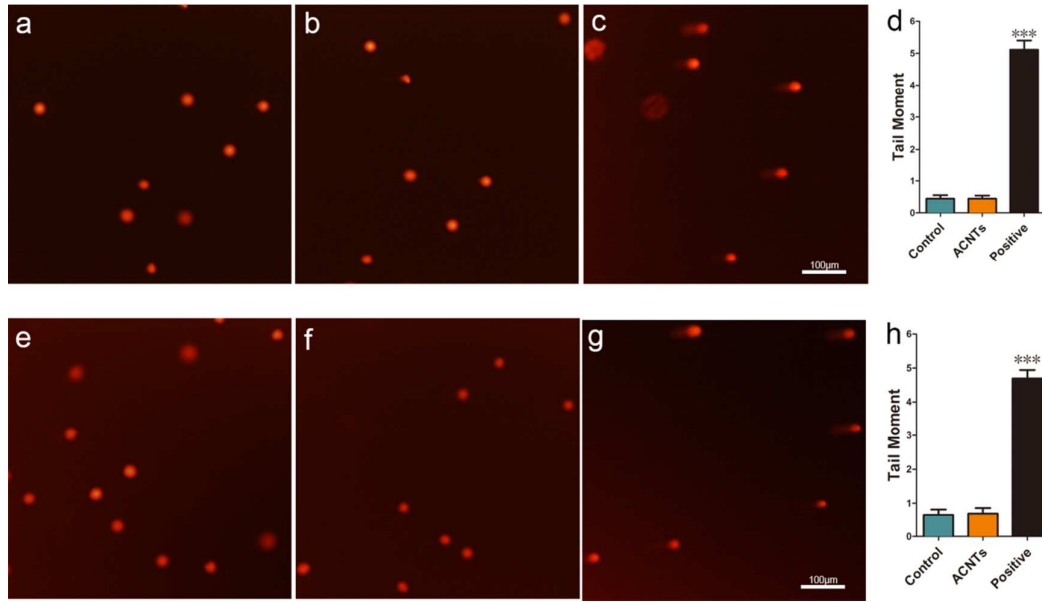


Figure S3. ACNTs did not induce DNA damage. DNA damage of (a) control group of HDF cells, (b) ACNTs group of HDF cells, (c) positive group of HDF cells (treated with 50 μ M hydrogen peroxide for 20 minutes at 4°C), (d) statistical data of HDF cells, (e) control group of NIH3T3 cells, (f) ACNTs group of NIH3T3 cells, (g) positive group of NIH3T3 cells (treated with 50 μ M hydrogen peroxide for 20 minutes at 4°C) and (h) statistical data of NIH3T3 cells MC3T3 cells were evaluated with comet assay. The data showed that there were no significant different between ACNTs group and control group. ***p<0.001.

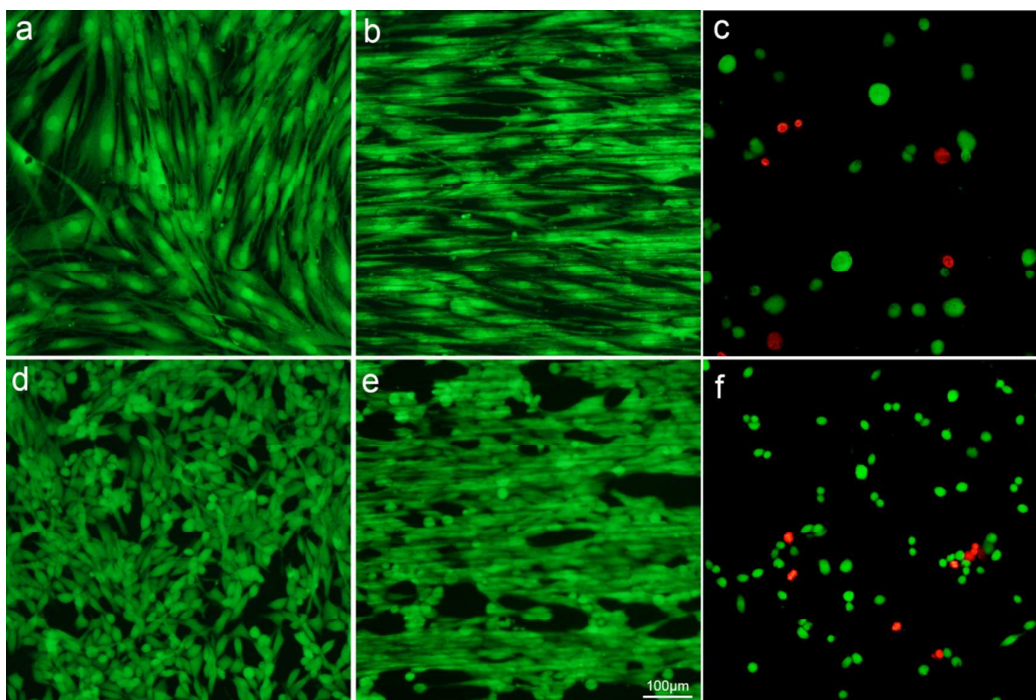


Figure S4. ACNTs did not induce cell death. Cell viability of (a) control group of HDF cells, (b) ACNTs group of HDF cells, (c) positive group of HDF cells (treated with 10 % dimethyl sulfoxide for 12 hours), (d) control group of NIH3T3 cells Positive group of HDF cells, (e) ACNTs group of NIH3T3 cells and (f) positive control of NIH3T3 cells (treated with 5 % dimethyl sulfoxide for 12 hours) were evaluated with cell death/live staining. The data showed that there were no significant different between ACNTs group and control group.

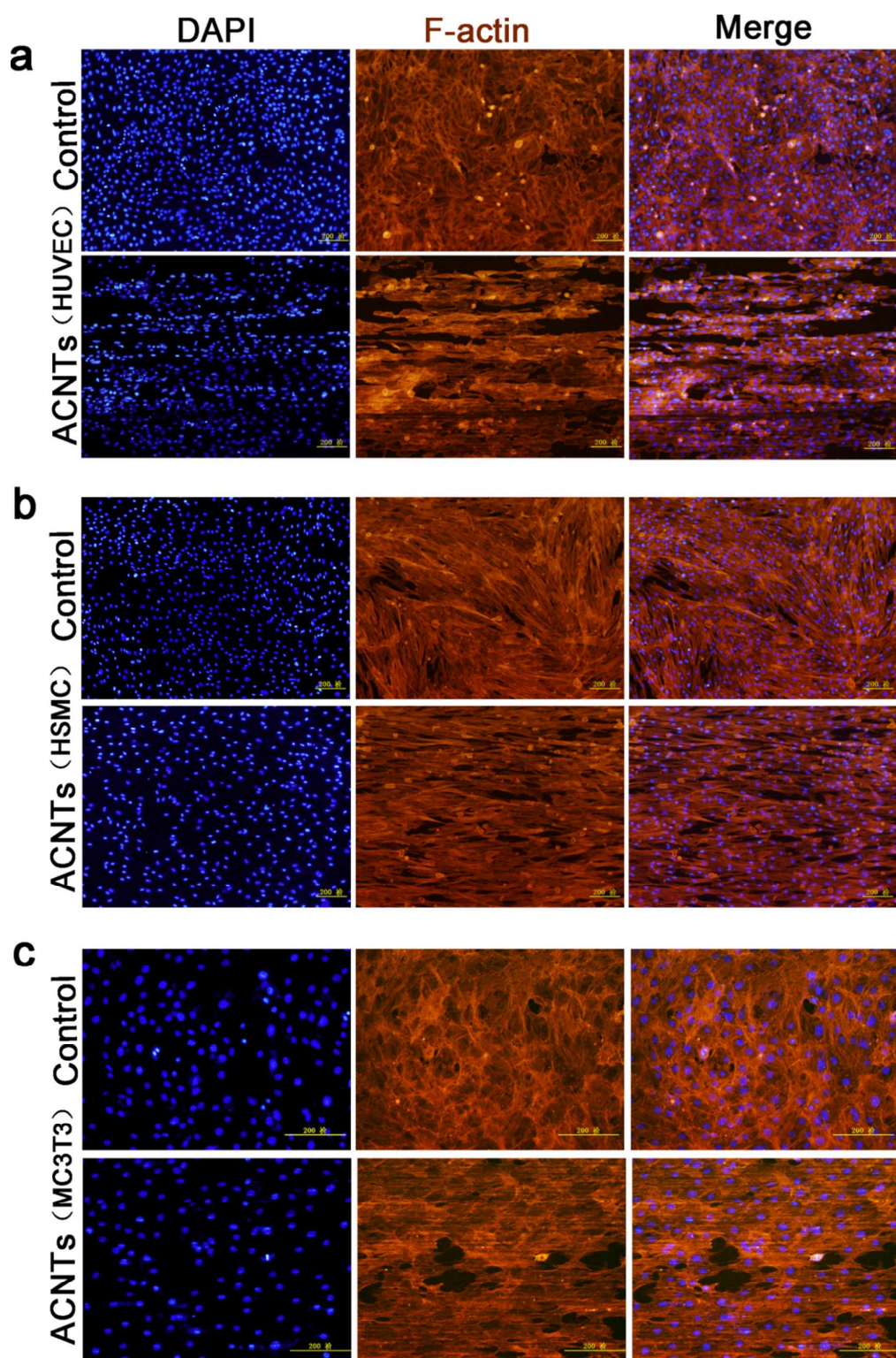


Figure S5. ACNTs directed (a) HUVEC cells, (b) HSMC cells, and (c) MC3T3 cells growth. (Blue: nucleus stained with DAPI; Red: cell cytoskeleton protein F-actin stained with phalloidin).

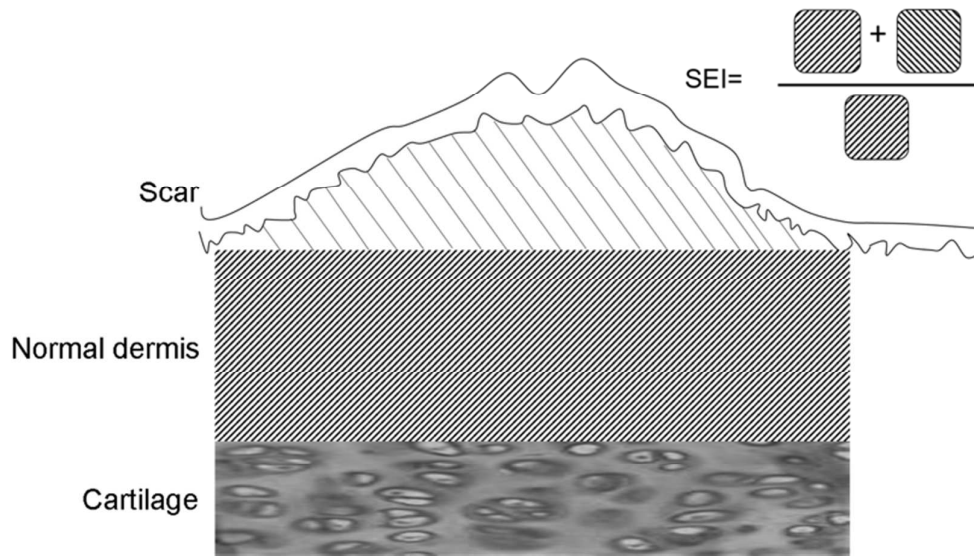


Figure S6 Schematic diagram of the SEI routine. SEI reflects an area ratio of the newly formed scar to the normal dermis.

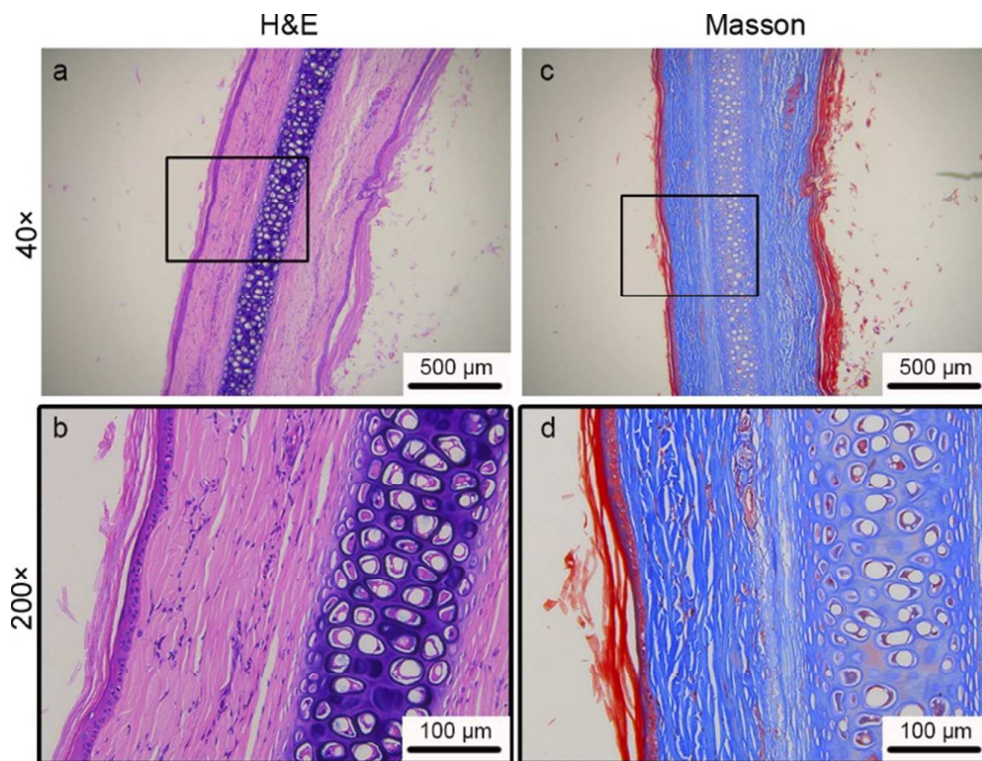


Figure S7 Histological evaluation of the normal group. (a, b) Stained with H&E. (c, d) Stained with Masson's. (b, d) The magnified photographs revealed that, in the normal dermal matrix, the fibrous tissue was arranged in strips and layers.

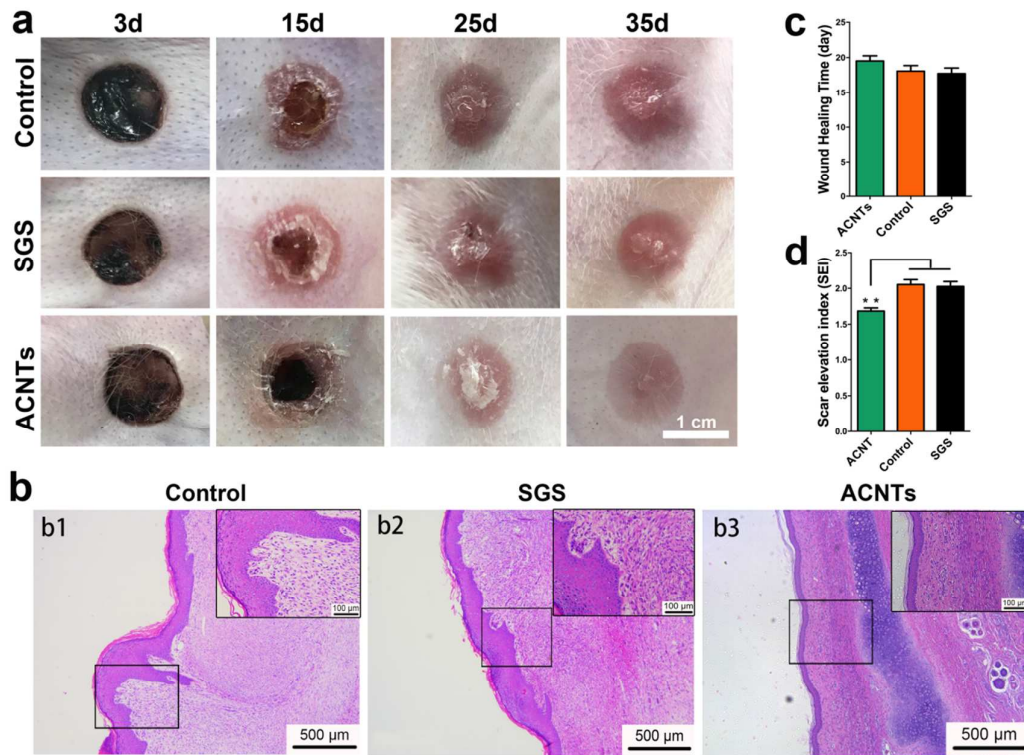


Figure S8 ACNTs work better than silicone gel sheet (SGS) during relatively short time. (a) Photographs of the wound during the healing procedure. (b) Histological appearance of scars harvested on Day 35 for ACNTs, control and SGS groups stained with H&E. (c) Comparison of the wound-healing time indicated no significant difference ($n=15$, $p > 0.05$). (d) Comparison on SEI. Dermal hypertrophy was measured by SEI on Day 35 according to the H&E stained sections ($n=15$, $**p < 0.01$).

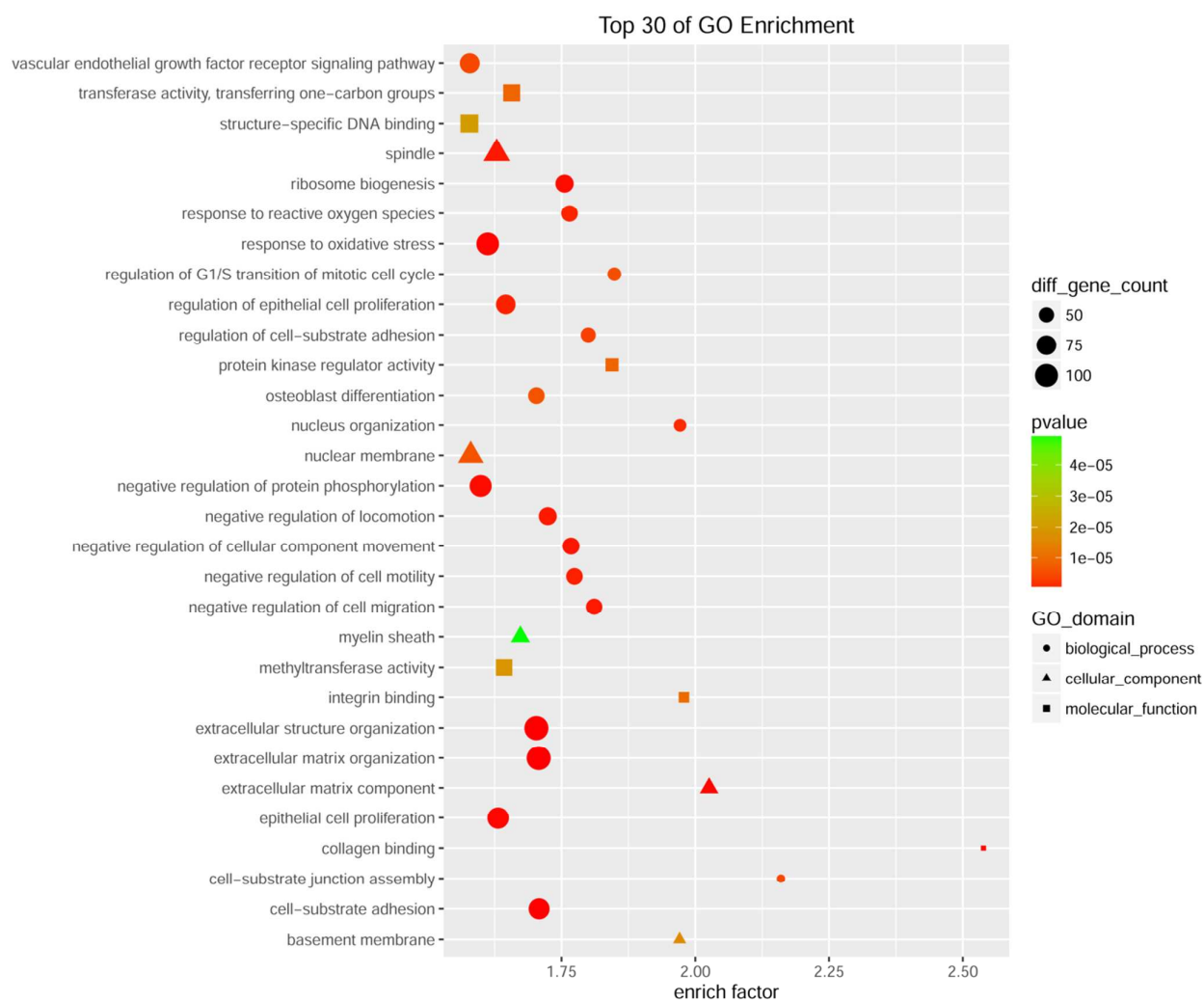


Figure S9. Top 30 categories of GO enrichment analysis. Size of the spots represented different genes count, the colour represented p -value and the shape of the spots represented gene type.

Supporting Information Table S1. Top 30 categories of GO enrichment analysis.

GO. id	Gene	Term	Count	p-value	Enrich factor
0005518	C1QTNF1/COMP/LRRC15/CHADL/DCN/DSPP/ECM2/NID2/ITGA11/FN1/ABI3BP/PCOLCE2/RELL2/ITGA1/ITGA2/ITGA3/LUM/S MAD3/SMAD4/NID1/COL5A3/HSD17B12/P3H1/SPARC/THBS1/TNXB/VWF/ANTXR1/ITGA10/SERPINH1	collagen binding	30	0.00029	2.5386
0007044	DLC1/FERMT2/WHAMM/CLASP2/CLASP1/FN1/CORO1C/GREM1/GPM6B/APOD/ITGA6/ITGB4/KDR/LAMA5/LAMB3/LAMC1/L AMC2/SMAD3/MMP14/PDPK1/PLEC/BCAS3/EPB41L5/PTPRK/BCL2/SDC4/SFRP1/DST/THBS1/THY1/ACVRL1/ROCK2/PPM1F	cell-substrate junction assembly	33	0.03427	2.1601
0044420	COL1A1/COL3A1/COL4A6/COL5A1/COL5A2/COL6A1/COL8A1/COL8A2/COL12A1/COL15A1/DCN/DLG1/FBLN1/FBN2/NID2/FN 1/ANG/RELL2/EFEMP2/ADAMTSL5/ITGA6/ITGB4/LAMA4/LAMA5/LAMB1/LAMB3/LAMC1/LAMC2/LOXL1/LOXL2/LUM/MFA P1/MFAP4/NID1/COL5A3/SERPINF1/P3H2/ERBB2IP/NTN4/SMOC1/SLC1A3/DST/SPARC/THBS4/TIMP3/TNXB/FRAS1/HMCN1/R UNX1/NTN1	extracellular matrix component	50	0.00015	2.0258
0005178	ADAM10/GPNMB/CIB2/ADAMTS5/NISCH/COL3A1/COL5A1/ECM2/EMP2/FBLN1/FN1/ICAM1/ICAM2/ICAM3/ITGA6/ITGA3/KD R/LAMA5/LAMB1/MFGE8/MMP14/NF2/ERBB2IP/DST/ADAM17/THBS1/THBS4/THY1/ICAM5/TNXB/VWF/JAM3/SEMA7A/PPAP 2B/ADAM23/WISP2/CD9/S1PR2	integrin binding	38	0.01544	1.9788
0006997	ZMPSTE24/CHMP4B/NUP35/DMPK/TOR1A/EMD/NUP205/PSME4/SYNE1/SUN1/PES1/NUP62/CNEP1R1/TOR1AIP1/CHMP2A/GP ER1/HIPK2/HMGB2/LMNA/NUP88/NUP98/GOLM1/PML/TMEM33/PPP2R2A/NDC1/ASUN/NUP107/CHMP1B/RPS19/SRPK1/SRPK 2/VRK1/NDEL1/SEH1L/ZPR1/CCNB1/PYGO2/CCNB2/NEK9/NOLC1/VPS4B/NUP93/NUP58/NUP153	nucleus organization	45	0.01411	1.9716
0005604	COL4A6/COL5A1/COL8A1/COL8A2/COL15A1/DLG1/FBLN1/NID2/FN1/ANG/RELL2/EFEMP2/ITGA6/ITGB4/LAMA4/LAMA5/LA MB1/LAMB3/LAMC1/LAMC2/LOXL1/LOXL2/NID1/SERPINF1/P3H2/ERBB2IP/NTN4/SMOC1/DST/SPARC/THBS4/TIMP3/FRAS1/ HMCN1/RUNX1/NTN1	basement membrane	36	0.01585	1.9707

2000045	CTDSP2/CTDSPL/CDKN1B/PDPN/E2F7/E2F1/EGFR/EZH2/MYO16/PSME4/KANK2/MTBP/RGCC/PRMT2/ID2/MUC1/PML/PID1/R FWD3/PRKDC/MEPCE/PSMA1/PSMA2/PSMA3/PSMA7/PSMB8/PSMB9/PSMB10/PSMD2/PSMD4/PSMD11/PSME1/CTDSP1/RB1/S ENP2/BCL2/RDX/BID/SMARCA4/ADAM17/TP53/UBC/CDC45/CUL4B/CUL4A/DGKZ/HACD1/PSMF1/KIF14	regulation of G1/S transition of mitotic cell cycle	49	0.04181	1.8487
0019887	SPRY2/CDKN1B/CDKN1C/CDKN2C/FGFR1OP/PKIG/CISH/CKS1B/SOCS4/HEXIM2/ANKRD54/ERCC6/FGF13/IBTK/GAS6/GREM 1/RGCC/HSPB1/IGF2/NCK1/NPM1/PRKAG2/MBIP/CDK5RAP1/CAB39/PIK3CA/FAM20A/PARP16/ELP3/CAMK2N1/PKIA/MAP2K 1/CCNL1/RAC2/STK4/WNT11/DBF4B/CALM2/CALM3/CASP3/SOCS1/IQGAP1/SOCS2/CCNE2/FAM58A/STRADA/SOCS5	Protein kinase regulator activity	47	0.01354	1.8445
0030336	ADA/HDAC5/RNF41/SEMA3A/DLC1/ADARB1/ABHD2/NISCH/COL3A1/DAB2IP/CYP1B1/VASH1/CLASP2/CLASP1/SRGAP2/GTP BP4/CORO1C/CLIC4/GREM1/GNRH1/SCAI/RGCC/APOD/APOE/IL1RN/SVBP/ARHGAP4/ARHGDIB/NF1/NF2/SERPINE1/EGFL7/ EVL/SERPINF1/MAP2K5/PTGER4/ABHD6/PTPRG/PTPRK/PTPRM/FAM60A/BCL2/CCL2/CXCL12/SFRP1/NDRG4/STAT3/STC1/B ST2/THBS1/THY1/TPM1/C5/WNT11/IFITM1/C16orf45/IL33/NOG/SLC9A3R1/ACVRL1/TP53INP1/MAGI2	negative regulation of cell migration	62	0.00573	1.8110
0010810	CDK6/DLC1/CCR7/COL1A1/COL8A1/CSF1/DNM2/ECM2/EMP2/FBLN1/CLASP2/CLASP1/FN1/CORO1C/FAM21C/ABI3BP/GREM 1/DISC1/GPM6B/RELL2/APOD/ITGA3/KDR/ARL2/SMAD3/MFI2/MMP14/NF1/NF2/NID1/NINJ1/SERPINE1/HSD17B12/VIT/PIK3R 1/PLAU/BCAS3/LRRC16A/PTN/EPB41L5/RAC2/BCL2/RSU1/SDC4/SFRP1/THBS1/C1QBP/VEGFC/FZD4/MYADM/HACD1/ACVRL 1/ROCK2/PPM1F/CDC42	regulation of cell-substrate adhesion	55	0.02903	1.8001
2000146	ADA/HDAC5/RNF41/SEMA3A/DLC1/ADARB1/SPINT2/ABHD2/NISCH/COL3A1/DAB2IP/CYP1B1/FBLN1/VASH1/CLASP2/CLAS P1/SRGAP2/GTPBP4/CORO1C/CLIC4/GREM1/GNRH1/SCAI/RGCC/APOD/APOE/IL1RN/SVBP/ARHGAP4/ARHGDIB/NF1/NF2/SE RPINE1/EGFL7/EVL/SERPINF1/MAP2K5/PTGER4/ABHD6/PTPRG/PTPRK/PTPRM/FAM60A/BCL2/CCL2/CXCL12/SFRP1/NDRG4/ STAT3/STC1/BST2/THBS1/THY1/TPM1/C5/WNT11/IFITM1/C16orf45/IL33/NOG/SLC9A3R1/ACVRL1/TP53INP1/MAGI2	negative regulation of cell motility	64	0.00864	1.7740

0051271	ADA/HDAC5/RNF41/CDKN1B/SEMA3A/DLC1/ADARB1/SPINT2/ABHD2/NISCH/COL3A1/DAB2IP/CYP1B1/FBLN1/VASH1/CLASP2/CLASP1/SRGAP2/GTPBP4/CORO1C/CLIC4/GREM1/GNRH1/SCAI/RGCC/APOD/APOE/IL1RN/SVBP/ARHGAP4/ARHGDIB/NF1/NF2/SERPINE1/EGFL7/EVL/SERPINF1/MAP2K5/PTGER4/ABHD6/PTPRG/PTPRK/PTPRM/FAM60A/BCL2/CCL2/CXCL12/SFRP1/NDRG4/STAT3/STC1/BST2/TGFBR3/THBS1/THY1/TPM1/C5/WNT5A/WNT11/IFITM1/C16orf45/SEMA5A/IL33/NOG/SLC9A3R1/ACVRL1/TP53INP1/MAGI2	negative regulation of cellular component movement	68	0.00470	1.7677
0000302	ADA/HDAC6/PPIF/TXNIP/CCR7/COL1A1/PPARGC1B/ROMO1/CYP1B1/DHFR/NQO1/DNM2/ENDOG/ERCC6/EZH2/FANCC/FKBP1B/SETX/FOXO1/FOS/FXN/PRDX5/GPX7/GUCY1B3/ERO1A/HBA2/HSPD1/APOD/APOE/IL1R1/AQP1/JUN/AREG/KPNA4/MMP3/MPV17/MTR/GPX8/PRDX1/GLRX2/PDGFRA/PDGFBR/PDK2/STK26/ATP7A/CYCS/APTX/MAPK7/PTPRK/PYCR1/PLEKHA1/BCL2/SDC1/PINK1/BMP4/BNIP3/SOD2/SOD3/THBS1/TPM1/NR4A3/FOSL1/CASP3/AKR1C3	response to reactive oxygen species	64	0.01052	1.7650
0042254	RCL1/NOP56/WDR3/EBNA1BP2/ERI2/WBSCR22/DIS3L/FTSJ3/RPL10L/SRFBP1/FASTKD2/DIS3/DHX30/PDCD11/RRP1B/RRS1/BOP1/EXOSC2/PES1/GTPBP4/DCAF13/ZNF658/GNL3/DIMT1/UTP20/GNL2/GTF2H5/SNU13/NOP2/NPM1/NUP88/GEMIN4/EXOSC3/MRPS7/RRNAD1/UTP18/UTP11L/MRTO4/NOP16/NOP58/YBEY/SETD4/DDX56/WDR55/HEATR1/RNMTL1/LSG1/CHD7/NHP2/TSR1/UTP6/EXOSC5/UTP3/RNASEL/RPL23A/RPS17/RPS19/RPS27/SURF6/BYSL/C1QBP/XPO1/EFTUD1/RBFA/RPF1/LAS1L/UTP15/MPV17L2/CIRH1A/NOP14/NOLC1/UTP14C/EIF4A3/BMS1	ribosome biogenesis	74	0.00207	1.7556
0040013	ADA/HDAC5/RNF41/SEMA3A/DLC1/ADARB1/SPINT2/ABHD2/NISCH/COL3A1/DAB2IP/CYP1B1/FBLN1/VASH1/CLASP2/CLASP1/SRGAP2/GTPBP4/CORO1C/CLIC4/GREM1/GNRH1/SCAI/RGCC/APOD/APOE/IL1RN/ITGAV/SVBP/ARHGAP4/ARHGDIB/MRE11A/NF1/NF2/SERPINE1/EGFL7/EVL/SERPINF1/MAP2K5/PTGER4/ABHD6/PTPRG/PTPRK/PTPRM/PTX3/FAM60A/BCL2/ROBO2/CCL2/CXCL12/SFRP1/NDRG4/STAT3/STC1/BST2/THBS1/THY1/TPM1/C5/WNT5A/WNT11/TRIM25/TRIM26/IFITM1/FCN3/C16orf45/SEMA5A/IL33/NOG/SLC9A3R1/ACVRL1/TP53INP1/MAGI2	negative regulation of locomotion	73	0.00540	1.7244

0031589	CDK6/DLC1/FERMT2/BVES/CCR7/WHAMM/COL1A1/COL3A1/COL8A1/COL13A1/CSF1/DNM2/ECM2/EMP2/FBLN1/NID2/ITGA11/CLASP2/CLASP1/FN1/SRGAP2/CORO1C/FAM21C/ABI3BP/SNED1/GAS6/TIMM10B/GREM1/LYPD3/DISC1/GPM6B/RELL2/ID1/APOD/ITGA6/ITGA1/ITGA2/ITGA3/ITGAV/ITGB4/KDR/LAMA5/LAMB1/LAMC1/ARL2/SMAD3/SMAD6/MF12/MKLN1/MMP14/NF1/NF2/NID1/NINJ1/COL5A3/SERPINE1/HSD17B12/PDPK1/TMEM8B/VIT/PIK3R1/PLAU/PPARD/BCAS3/LRRC16A/PARVA/PTN/EPB41L5/PTPRK/RAC2/BCL2/RSU1/SDC4/SFRP1/RAPH1/THBS1/THY1/C1QBP/TNXB/VEGFC/VWF/PEAK1/FZD4/JAM3/ANTXR1/ITGA10/SGCE/MYADM/HACD1/ACVRL1/ROCK2/PPM1F/KIF14/CDC42	cell-substrate adhesion	94	0.00024	1.7080
0030198	ADAM8/ADAM10/CRTAP/PRDX4/SPINT2/RIPK3/ADAMTS5/EMILIN1/COL1A1/COL3A1/COL4A6/COL5A1/COL5A2/COL6A1/COL8A1/COL8A2/COL9A2/COL9A3/COL10A1/COL12A1/COL13A1/COL15A1/COMP/VCAN/CHADL/CYP1B1/DCN/OLFML2A/DPP4/DSPP/ECM2/FBLN1/FBN2/EFEMP1/NID2/ITGA11/CLASP2/FMOD/CLASP1/FN1/NCSTN/ABI3BP/GAS6/GREM1/B4GALT1/GPM6B/RGCC/ERO1A/EFEMP2/HSP90AB1/APLP2/ICAM1/ICAM2/ICAM3/ITGA6/ITGA1/ITGA2/ITGA3/ITGAV/ITGB4/KDR/LAMA4/LAMA5/LAMB1/LAMB3/LAMC1/LAMC2/LGALS3/LOX/LOXL1/LOXL2/LTBP3/LUM/SMAD3/MFAP1/MFAP3/MFAP4/MF12/MMP3/MMP7/MMP12/MMP14/MMP19/MPV17/NCAM1/NF1/NID1/TNFRSF11B/COL5A3/SERPINE1/F11R/HSD17B12/VIT/PLEC/ATP7A/ADAMTSL4/P3H2/CSGALNACT1/SULF2/PTPRS/JAM2/NTN4/SDC1/SDC4/P3H1/BMP4/DST/SPARC/ADAM17/THBS1/TNXB/VWF/PXDN/GFOD2/COL21A1/GDF5/CASP3/JAM3/RECK/ITGA10/SERPINH1	extracellular matrix organization	121	2.72E-06	1.7075
0043062	ADAM8/ADAM10/CRTAP/PRDX4/SPINT2/RIPK3/ADAMTS5/EMILIN1/COL1A1/COL3A1/COL4A6/COL5A1/COL5A2/COL6A1/COL8A1/COL8A2/COL9A2/COL9A3/COL10A1/COL12A1/COL13A1/COL15A1/COMP/VCAN/CHADL/CYP1B1/DCN/OLFML2A/DPP4/DSPP/ECM2/FBLN1/FBN2/EFEMP1/NID2/ITGA11/CLASP2/FMOD/CLASP1/FN1/NCSTN/ABI3BP/GAS6/GREM1/B4GALT1/GPM6B/RGCC/ERO1A/EFEMP2/HSP90AB1/APLP2/ICAM1/ICAM2/ICAM3/ITGA6/ITGA1/ITGA2/ITGA3/ITGAV/ITGB4/KDR/LAMA4/LAMA5/LAMB1/LAMB3/LAMC1/LAMC2/LGALS3/LOX/LOXL1/LOXL2/LTBP3/LUM/SMAD3/MFAP1/MFAP3/MFAP4/MF12/MMP3/MMP7/MMP12/MMP14/MMP19/MPV17/NCAM1/NF1/NID1/TNFRSF11B/COL5A3/SERPINE1/F11R/HSD17B12/VIT/PLEC/ATP7A/ADAMTSL4/P3H2/CSGALNACT1/SULF2/PTPRS/JAM2/NTN4/SDC1/SDC4/P3H1/BMP4/DST/SPARC/ADAM17/THBS1/TNXB/VWF/PXDN/GFOD2/COL21A1/GDF5/CASP3/JAM3/RECK/ITGA10/SERPINH1	extracellular structure organization	121	3.26E-06	1.7031

0001649	HDAC5/ALYREF/CDK6/GPNMB/SYNERIP/CEBPB/MYBBP1A/CTHRC1/TWIST2/COL1A1/COL6A1/VCAN/DHX9/FBN2/FHL2/ITGA11/ZHX3/UFL1/HEY1/GTPBP4/GLI3/HSD17B4/HSPE1/IARS/TMEM119/ID1/ID2/ID3/IL6R/IL6ST/AREG/NBR1/SMAD3/SMAD5/NF1/NPPC/ATP5B/ATRAID/HDAC7/PHB/FAM20C/CTNBP1/PTCH1/IFT80/RDH14/BGLAP/SMOC1/SFRP1/BMP4/BMPR1A/WNT11/AXIN2/SEMA7A/IFITM1/RUNX2/CBFB/ACVR1/ACVR2A/NOG/ACVR2B/HAND2/HDAC4/RASSF2	osteoblast differentiation	63	0.04786	1.7029
0043209	ACTR1A/TUBA1B/TUBB4B/NDRG1/CCT2/STIP1/DLG1/DLST/DPYSL2/EEF1A1/ENO2/ERBB2/NFASC/SCRIB/SERINC5/CNRIP1/GLUL/GNB1/SLC25A4/SLC25A5/PDIA3/EHD3/HSPA2/HSPA9/HSP90AA1/HSPD1/MYO1D/ATP1A2/ACO2/CLDN11/PRDX1/ATP5B/PLLP/PDHA1/PHB/PITPNA/BCL2/RDX/EXOC4/TCP1/THY1/CCT3/UCHL1/UQCRRS1/VDAC1/VDAC2/JAM3/CNTNAP1/NAPG/NA/COX5A/CD59/CDC42	myelin sheath	53	0.04718	1.6731
0016741	PRMT3/PRMT5/WDR5/PRDM5/PRDM4/INMT/HENMT1/WBSCR22/SMYD4/TRMT61A/FTSJ3/COMTD1/MTFMT/CARNMT1/METTL21A/TRMT44/ALDH1L2/DNMT1/EEF2KMT/EZH2/BHMT2/FTSJ1/METTL7A/METTL21B/GART/GCSH/DIMT1/AMT/N6AMT1/HNMT/PRMT2/METTL2A/MGMT/MTR/ATIC/NNMT/NOP2/RRNAD1/PCMT1/LCMT1/METTL13/COQ3/SETD4/PRMT7/NSUN2/TRMT10C/RNMTL1/PCMTD2/NDUFAF7/TRMU/KMT2E/MEPCE/METTL3/METTL14/SETMAR/SHMT1/METTL17/SMYD3/FAM86B2/TPMT/PRDM2/NDUFAF5/METTL8/SETD7/EED/RNMT/METTL18	transferase activity, transferring one-carbon groups	67	0.01356	1.6568
0050678	CDK6/CDKN1B/CDKN1C/FRS2/OSR2/TWIST2/CD109/DAB2IP/CYBA/DLG1/ECM1/EGFR/ERBB2/FGF7/FGFR3/VASH1/B4GALT1/GLUL/ANG/RGCC/HMGB2/ID1/APOE/JUN/KDR/LAMB1/LAMC1/SMAD3/MMP12/MYC/NF1/NFIB/IFT52/EGFL7/SERPINF1/PGF/PLAU/PPARD/AGGF1/LIMS2/MAP2K5/PTCH1/IFT80/WDR48/PTN/PTPRK/PTPRM/WFDC1/RB1/SAV1/CCL2/CXCL12/SFRP1/SIX1/BMP4/BMPR1A/SPARC/BRCA2/STAT1/KLF9/TBX1/TGFBF3/THBS1/THBS4/VDR/VEGFB/VEGFC/WNT5A/NR4A3/ZNF703/GDF5/CAV1/CAV2/TNFSF12/NRP2/SEMA5A/PYGO2/NOG/ACVRL1/CYP7B1/MAGED1/MTSS1/CDC42	regulation of epithelial cell proliferation	83	0.00849	1.6457

0008168	PRMT3/PRMT5/WDR5/PRDM5/PRDM4/INMT/HENMT1/WBSCR22/SMYD4/TRMT61A/FTSJ3/COMTD1/CARNMT1/METTL21A/T RMT44/ALDH1L2/DNMT1/EEF2KMT/EZH2/BHMT2/FTSJ1/METTL7A/METTL21B/GART/GCSH/DIMT1/AMT/N6AMT1/HNMT/P RMT2/METTL2A/MGMT/MTR/NNMT/NOP2/RRNAD1/PCMT1/LCMT1/METTL13/COQ3/SETD4/PRMT7/NSUN2/TRMT10C/RNMT L1/PCMTD2/NDUFAF7/TRMU/KMT2E/MEPCE/METTL3/METTL14/SETMAR/METTL17/SMYD3/FAM86B2/TPMT/PRDM2/NDUF AF5/METTL8/SETD7/EED/RNMT/METTL18	methyltransferase activity	64	0.02875	1.6426
0050673	CDK6/CDKN1B/CDKN1C/CEBPB/FRS2/CHUK/OSR2/TWIST2/COL8A1/COL8A2/CD109/DAB2IP/CYBA/BMPER/DLG1/ECM1/EG FR/ERBB2/FGF7/FGFR3/VASH1/NCSTN/B4GALT1/GLUL/ANG/RGCC/HMGB2/ID1/ID2/APOE/IGFBP4/JUN/AREG/KDR/KIT/LA MB1/LAMC1/LOXL2/SMAD3/MMP12/MMP14/MYC/NF1/NFIB/SIDT2/IFT52/EGFL7/SERPINF1/PGF/PLAU/PPARD/AGGF1/FERM T1/LIMS2/MAP2K1/MAP2K5/PTCH1/IFT80/WDR48/PTN/PTPRK/PTPRM/WFDC1/RB1/SAV1/CCL2/CXCL12/SFRP1/SIX1/BMP4/B MPR1A/SPARC/BRC2/STAT1/KLF9/TBX1/TGFBF3/THBS1/THBS4/VDR/VEGFB/VEGFC/WNT5A/NR4A3/ZNF703/GDF5/CAV1/ CAV2/TNFSF12/NRP2/SEMA5A/PYGO2/ACVR2A/NOG/ACVRL1/CYP7B1/SCARB1/MAGED1/MTSS1/CDC42	epithelial cell proliferation	100	0.00113	1.6315
0005819	FRY/TADA3/SPAG5/CENPF/CBX1/KATNA1/CBX3/CCSAP/DDX11/DIAPH1/EML1/CENPV/SKA3/CEP162/MAPRE1/TPX2/CLASP 2/SEPT6/TTC28/CLASP1/NUP62/KIF4A/POC1A/GEM/INVS/PRPF19/WDR62/RACGAP1/SLC25A5/ANXA11/HSPB1/INCENP/KIF22 /ARL3/MAD2L1/MAP4/MZT1/MYH9/NEDD9/NEK2/NPM1/ODF2/HAUS6/NSUN2/SPDL1/HAUS4/CDC48/HAUS7/ACOT13/MAP2 K5/FMN2/KLHL42/RANGAP1/RB1/AGBL5/RPS6KA2/CEP85/AURKA/TTK/TUBG1/VRK1/PTP4A1/EVI5/AUNIP/MAP7D3/TBL1X R1/CALM2/CALM3/NDEL1/FAM83D/FAM110A/CUL3/KBTBD8/TNKS/CDC16/CCNB1/CEP95/HAUS8/KIF23/VPS4B/KIF20B/ESP L1/SEPT7/KLHL21/CDC20/KIF14/CDC27/CDC42	spindle	88	0.00070	1.6288
0006979	ADA/HDAC6/PPIF/COQ7/RCAN2/ARL6IP5/TXNIP/LIAS/PSIP1/CHUK/CCR7/COL1A1/CPEB2/PPARGC1B/SRXN1/ROMO1/CYP1B 1/DHFR/NQO1/DNM2/TOR1A/ENDOG/STX2/ERCC1/ERCC6/EZH2/FANCC/DDIAS/ALDH3B1/FKBP1B/MSRB2/PLA2R1/SETX/FO XO1/FOXO3/FOS/SLC7A11/FXN/MSRB3/PRDX5/GCLC/GPX4/GPX7/GUCY1B3/ERO1A/HBA2/HSPB1/HSPD1/IDH1/APOD/APOE/ IL1R1/AQP1/JUN/AREG/KPNA4/MGMT/MMP3/MMP14/MPV17/MTR/NDUFA6/NONO/SLC11A2/GPX8/PRDX1/GLRX2/PDGFRA/ PDGFRB/PDK2/STK26/PML/ATP7A/CYCS/APTX/OXR1/NDUFA12/MAPK7/MAP2K1/PTGS1/PTPRK/PYCR1/PLEKHA1/BCL2/SD C1/SEPP1/PINK1/BMP4/BNIP3/SOD2/SOD3/THBS1/TP53/TPM1/UCN/PXDN/NEIL1/NR4A3/FOSL1/CASP3/CHD6/STC2/AKR1C3/P NPT1/NOL3/PDLIM1/LONP1/TP53INP1	response to oxidative stress	108	0.00072	1.6120

0001933	CTDSP2/CTDSPL/SPRY1/SPRY2/CDKN1B/CDKN1C/CDKN2C/ADARB1/ZMYND11/FGFR1OP/PKIG/DUSP10/CISH/FAM129A/SOCS4/HEXIM2/CD109/PAQR3/DAB2IP/DLG1/DUSP5/DUSP6/MECOM/FBLN1/DKK1/FOXO1/SIRT3/CORO1C/NUP62/IBTK/PYDC1/PTPN22/GREM1/CHORDC1/PDCD4/RAPGEF1/PYCARD/HMGCR/HSPB1/IGBP1/APOE/IGF1R/JUN/ARRB1/SMAD3/SMAD6/MYC/NCK1/NF1/NF2/NPM1/P2RX7/ATRAID/PRKAG2/PDE4D/MBIP/CDK5RAP1/ENPP1/PDPK1/PER1/PHB/DNAJC10/DDIT4/PID1/CA MK2N1/PK1A/PRKDC/PSEN2/PTPN1/ZFYVE28/CTDSP1/SNX6/RB1/RGS3/BDKRB1/BDKRB2/SFRP1/SMYD3/PINK1/BMP4/THY1/UCHL1/CALM2/CALM3/SPRY4/CASP3/SNX25/ZBED3/NCK2/CUL3/UBASH3B/CAV1/PPAP2B/SOCS1/SOCS2/CCNB1/DNAJA3/MYADM/NOG/SLC9A3R1/PPM1F/SOCS5/RASSF2	negative regulation of protein phosphorylation	103	0.00219	1.5988
0031965	LRPPRC/ZMPSTE24/WDR3/CBX3/DTX2/OSBPL6/CMTM3/TBC1D20/NUP35/FAM76B/PRICKLE1/ADRA1B/QSOX2/DHCR7/DMPK/TOR1A/EGFR/EMD/WDFY3/NUP205/ATP11B/DPY19L1/SYNE1/SUN1/COTL1/GTPBP4/NUP62/CERS6/CNEP1R1/ZZZ3/TOR1AIP1/GCHFR/DNAJC2/DPY19L4/SCAI/TMEM176B/ANXA4/AQP1/IL15RA/PNPLA7/LMNA/SMAD3/MX1/NUP98/P2RX4/P2RX5/P2RX7/MRTO4/NBAS/PML/TMEM38B/DDX19A/NDC1/ERBB2IP/PSEN2/NUP107/PTGDS/RANGAP1/SEN2/BCL2/RPS6KA2/SORT1/DNAJC1/AEN/RBM15/XPO1/SLC30A1/MAPKAPK3/CUEDC2/FAM188A/TSGA10/BRIP1/KAT8/TNRC18/LMNB2/TNKS/INA/WTAP/NUP93/SCRN1/NUP58/NUP153	nuclear membrane	82	0.00606	1.5803
0048010	AKAP9/RASA4/WASF2/BAIAP2/FRS2/PAQR3/DAB2IP/CYBA/DOCK1/HBEGF/DUSP5/DUSP6/EGFR/ERBB2/FGF5/FGF7/FGFR3/FGF/PSME4/FLT1/FYN/SHC2/RASGRP3/GRIN2D/HSPB1/HSP90AA1/ITGAV/JAK1/JAK3/JUP/KDR/KIT/MARK3/NCAM1/NCK1/NF1/NRAS/PDGFR/PDGFRB/PDPK1/PGF/PIK3CA/PIK3R1/SHC3/IL17RD/MAPK11/MAPK13/MAP2K1/PSMA1/PSMA2/PSMA3/PSMA7/PSMB8/PSMB9/PSMB10/PSMD2/PSMD4/PSMD11/PSME1/PTPN1/PTPRA/CCL2/RASAL3/SOS1/SPTBN1/UBC/VEGFB/VEGFC/MAPKAPK3/MAPKAP1/TMEM204/CALM2/CALM3/CAMK2G/FZD4/KBTBD7/NCK2/CUL3/CAV1/IRS2/NRP2/ROCK2/PSMF1/NRG2/RBX1/CDC42	vascular endothelial growth factor receptor signaling pathway	86	0.03572	1.5785

Supporting Information Table S2: Primers used for RT-PCR.			
Primers used for HDF cells (Human)		Primers used for NIH3T3 cells (Mouse)	
NFIB-F	CTGGCGTCTGGATCTAGTCAT	NFIB-F	CGTGCTGTGTCTTATCCA ATCC
NFIB-R	TGGAGGATTCTTGGCAGGAT	NFIB-R	TGTGATGTGGTGTGGCTG AA
GAPDH-F	TGGGCTACACTGAGCACCAG	GAPDH-F	CAATGAATAGGGCTACA GCA
GAPDH-R	AAGTGGTCGTTGAGGGCAAT	GAPDH-R	AGGGAGATGCTCAGTGTT GG
NOG-F	GCCAGCACTATCTCCACATC	NOG-F	GCCAGCACTATCTACACA TCC
NOG-R	AGCAGCGTCTCGTTCAGA	NOG-R	AGCAGCGTCTCGTTCAGA
CYP7B1-F	CGACTGTCCTCATATTCAACCA	CYP7B1-F	AGAAGTTCAGCAGCCGA TTATC
CYP7B1-R	ACGGCATTAGGTAACACTTCAG	CYP7B1-R	AATACCAGTGAGCCACA GAATG
PDPN-F	CCGAAGATGATGTGGTGACTC	PDPN-F	GACCGTGCCAGTGTGTGTT C
PDPN-R	CGATGCGAATGCCTGTTACA	PDPN-R	GTGCCTTGCCAGTAGATT CATT
SPAG16-F	CCATAACTGAAGCATCTGAAG	SPAG16-F	TTCTTCTCACCCTTCTG
SPAG16-R	AGTATCTGTAGCCTGTTCTTG	SPAG16-R	ATGGACACGATTGGAAT GA
ARHGAP26-F	TTGCTGAGTGCCTGATG	ARHGAP26-F	GGACAGTATCGGCTTCAG
ARHGAP26-R	ATTCTGCTTGTTGTTAG	ARHGAP26-R	CATTCTCAGGTAGGTCTT CA
MYOZ2-F	TGGATGGAAGTAACTTGGA	MYOZ2-F	ACATCGCACCAGGATATT C
MYOZ2-R	CCTGGAGCAATGTTGTCT	MYOZ2-R	TCTGTCAGCAGCAGTAGT
TNS4-F	CATCCATCACCAACTCCAT	TNS4-F	ACCTTATCCGTCACTTCC T

TNS4-R	TGTCCATCACGAACTTCAT	TNS4-R	CTCCACACTCACAGAACT C
KCNAB2-F	AGTGGCTGAAGGACAAGA	KCNAB2-F	CAGTGGTTGAAGGACAA GA
KCNAB2-R	CAATCTCGTGGATAATGGAAG	KCNAB2-R	GACGATGGAAGACGACA A
COL1A1-F	GTGACGAGACCAAGAACT	COL1A1-F	GTGGCGGTTATGACTTCA
COL1A1-R	CTCATCATAGCCATAAGACAG	COL1A1-R	CTGCGGATGTTCTCAATC T
COL3A1-F	CTACGGCAATCCTGAACTT	COL3A1-F	CCTTCTACACCTGCTCCT
COL3A1-R	GCAACCATCCTCCAGAAC	COL3A1-R	CCACTCCAGACTTGACAT C
COL5A1-F	ACTTGCCTGATGGAATAACA	COL5A1-F	GAAGATTACGAAGTTCCT CAG
COL5A1-R	CCTGCTCGTTGTAGATGG	COL5A1-R	TAGTGCTCACAGTAGTCA TAG
COL15A1-F	TCACAGCATTAGCAACA	COL15A1-F	GATGGATGAAGTCGCTG AA
COL15A1-R	TTCCAGCCATCTCTAACAC	COL15A1-R	GGCAGGAGAGGATGAAG T
F: forward primer; R: reverse primer.			

Materials and methods

Materials

Double distilled water. Dulbecco's Modified Eagle Media (DMEM), Fetal bovine serum (FBS), Alexa Fluor® 555 Phalloidin, HRP-conjugated mouse and rabbit secondary antibodies and Trizol were purchased from Invitrogen (Thermo Fisher, CA, USA). Cell-light EdU DNA cell proliferation kit was purchased from RiboBio (Guangzhou, China). PrimeScript RT reagents, Kit and SYBR Premix Ex Taq, were purchased from Takara Biotechnology (Dalian, China). Annexin V-FITC Apoptosis Detection Kit was obtained from eBioscience (San Diego, CA, USA). The antibodies of caspase 3, caspase 8, Collagen I and anti-alpha tubulin antibody (Alexa Fluor® 647)

were purchased from Abcam (Cambridge, MA, USA). Antibodies to nuclear factor I B (NFIB), Noggin, cytochrome P450 family 7 subfamily B member 1 (CYP7B1), Amphiregulin, sperm associated antigen 16 (SPAG16), f, Myozenin 2, tensin 4 (TNS4), potassium voltage-gated channel subfamily A regulatory beta subunit 2 (KCNAB 2), Podoplanin, Collagen III and GAPDH were obtained from Proteintech. Anti-collagen type XV alpha 1 chain (anti-COL15A1) and anti-collagen type V alpha 1 chain (anti-COL5A1) were obtained from Sangon Biotech. Comet assay kit was purchased from Trevigen Inc (Gaithersburg, USA). Live/Dead Viability/Cytotoxicity Kit were purchased from Thermo Fisher Scientific (Shanghai, China). CICA-CARE Silicone gel sheet was purchased from Smith & Nephew (London, UK). The cell culture plates were all purchased from Corning Inc. and all other reagents were of an analytical grade.

Cell proliferation assay

ACNTs and glasses were placed in 24-well plates and sterilized with UV light for half an hour. About 1×10^4 cells were seeded in each well and cultured for two days. The images of cells of different time were captured using microscopy (IX81, Olympus) with 200 $\times$ magnification. Cell numbers were counted and analyzed using GraphPad Prism.

Cell apoptosis

Cells cultured on ACNTs or glass for three days were retrieved using trypsin and washed with PBS twice. The cells were first stained with Annexin V-FITC for 15 minutes at room temperature in the dark and followed by incubation with PI for 5 minutes in the binding buffer at room temperature in the dark. Finally, apoptosis was analyzed using Cell Lab Quanta SC (Beckman Coulter), and FLOWJO v7.6 software was applied for analyse of the data.

Single cell gel electrophoresis assay (comet assay)

Alkaline comet assay was performed adhering to the manufacturer's instruction. Briefly, about 2000 cells in PBS were mixed with LM Agarose at a ratio of 1:10 (v/v) and immediately pipetted 50 μ l onto CometSlideTM. Place slides at 4 °C in the dark for 30 minutes, and were immersed using Lysis Solution for 1 hour. Then immerse the

slide in Alkaline solution for 1 hour at 4 °C in the dark. The slides were run (21V) in running buffer for half an hour at 4 °C. The slides were then immersed in ddH₂O for twice and in 70% ethanol once. After being dried for 20 minutes, the slides were stained with ethidium bromide and viewed using microscopy (IX81, Olympus). Cells treated with 50 µM hydrogen peroxide for 20 minutes at 4°C were set as positive controls.

Cell live/dead staining

Cell live/dead staining was performed using LIVE/DEAD™ Viability/Cytotoxicity Kit. For the experiment, ACNTs and glasses were placed in 24-well plate and sterilized with UV light for half an hour. 2×10^4 cells were seeded into plates and cultured for two days. The staining reagent was prepared by adding 2.5µL calcein AM and 10µL ethidium homodimer-1 to 10mL DPBS. Then, we discarded the medium and added 400µL staining reagent. After stained for half an hour, the stained cells were observed under a microscope (IX81, Olympus). Cells treated with 10 % (v/v) dimethyl sulfoxide and with 5 % (v/v) dimethyl sulfoxide were set as positive control of HDF cells and NIH3T3 cells, respectively.

***In vivo* experiments**

Adult New Zealand rabbits, male, aged 3 months, were obtained from Slac Laboratory Animal Corporation. The 45 rabbits were randomly divided into three groups: ACNT, Silicone gel sheet (SGS) and Control groups ($n=15$). The animals were anaesthetized and hypertrophic scar models were established. Wounds of ACNTs group were covered with ACNTs sheets and adhesive bandages while the control group was covered with only adhesive bandages. For the SGS group, the wound was covered with only bandage in the early 25 days. On the 25th day (SGS must not be applied to an open wound), when complete wound healing was observed, SGS was applied to the wound and bandages were used to protect the wound area. The healing process was observed until rabbits were sacrificed on Day 35 after surgery. Afterwards, samples containing the full-thickness scar and the cartilage in the rabbit ear were collected for histological analysis.