Supplementary information

Nanosilver alleviates foreign body reaction and facilitates wound repair by regulating macrophage polarization

Chuangang YOU[1], Zhikang ZHU[1], Shuangshuang WANG[1,2], Xingang WANG[1], Chunmao HAN[1], Huawei SHAO[1]

[1]*Department of Burns & Wound Care Center, the Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou 310009, China*

[2]*Department of Burns, the First People's Hospital of Wenling, Wenling 317500, China*

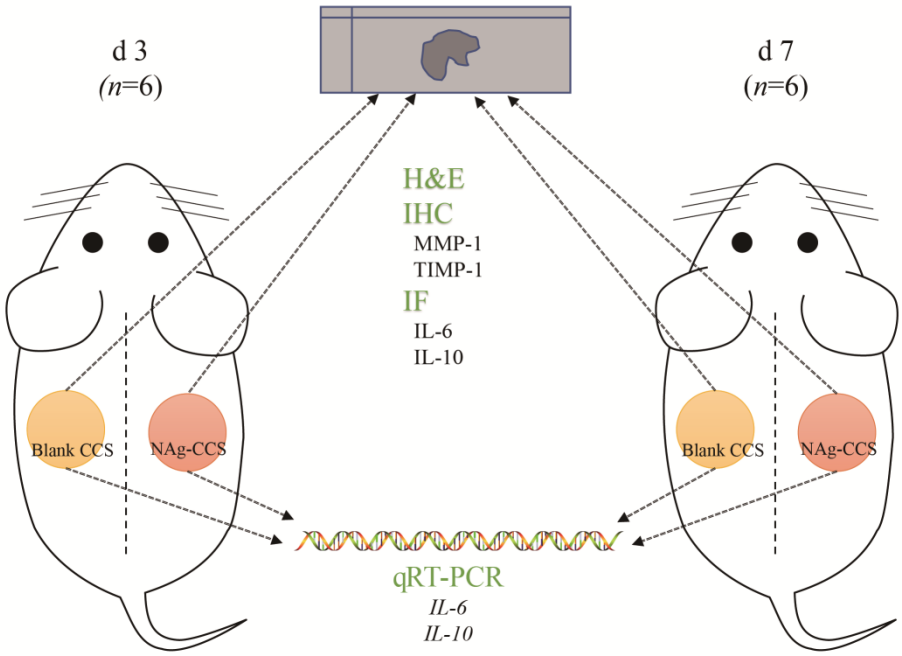

Fig. S1 Rat model experimental design. Nanosilver-loaded collagen-chitosan scaffolds (NAg-CCS) (one on one side and a blank CCS on the other) were implanted in specific-pathogen-free Sprague-Dawley rats, about 2 cm parallel from the spine. Rats from different groups were euthanized on postoperative Days 3 (*n*=6) and 7 (*n*=6), and the implants were harvested for subsequent experiments. Implant samples were fixed in 4% paraformaldehyde and processed for hematoxylin and eosin (HE) staining. Also, fragments were subjected to immunohistochemistry (IHC) with primary antibodies anti-matrix metalloproteinase 1 (MMP-1) or anti-metallopeptidase inhibitor 1 (TIMP-1). In contrast, others were processed for immunofluorescence (IF) with anti-interleukin 6 (IL-6) or anti-interleukin 10 (IL-10) primary antibodies. Meanwhile, frozen (−80 °C) samples were used to quantify the relative expression of *IL-6* and *IL-10* mRNA by qRT-PCR.

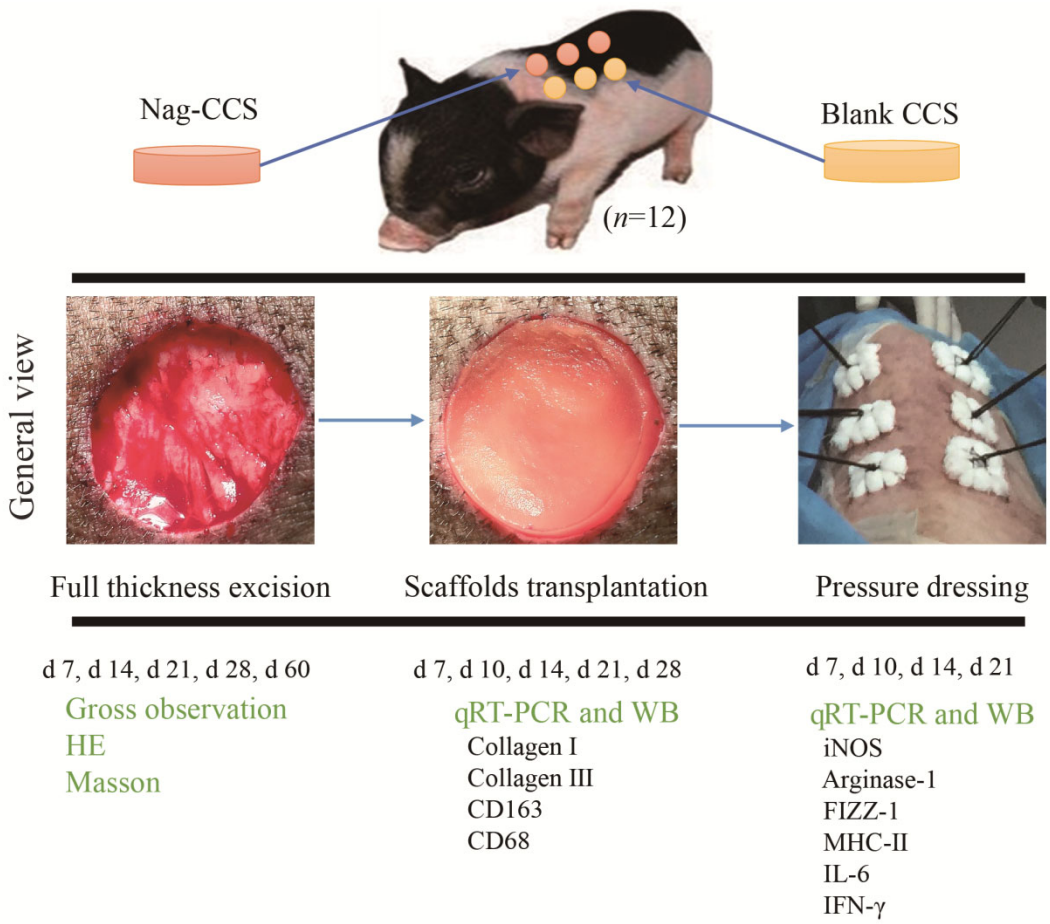

Fig. S2 Experimental design for wound transplantation of scaffolds in miniature pigs. NAg-CCSs and blank CCSs were transplanted in the backs of miniature pigs, about 4 cm parallel from the spine, three scaffolds on each side. Pigs from different groups were euthanized on postoperative Days 7, 10, 14, 21, 28, and 60, and skin samples were harvested for subsequent experiments. Implant samples were fixed in 4% paraformaldehyde and processed for hematoxylin and eosin (HE) and Masson staining. Also, fragments were subjected to analysis of Collagen I, Collagen III, CD163, and CD68 on Days 7, 10, 14, 21, and 28. Frozen (−80 °C) samples were also used to quantify the relative mRNA expression of *iNOS*, arginase-1 (*ARG1*), *FIZZ-1*, *MHC-II*, *IL-6*, and *IFN-γ* by qRT-PCR on Days 7, 10, 14, and 21.

Table S1 Primer sequences for quantitative reverse transcription qRT-PCR of rat samples

Gene	Primer sequence*
Rat *GADPH*	F: GATGCTGGTGCTGAGTATGRCG R: GTGGTGCAGGATGCATTGCTCTGA
Rat *IL-6*	F: ATGAAGTTTCTCTCCGCAAGAGACT R: TACTTCAAAGAGAGGCGTTCTCTGA
Rat *IL-10*	F: ATGCCTGGCTCAGCACTGCTATGTT R: TACGGACCGAGTCGTGACGATACAA

*Designed and synthesized by Sangon Biotech (Shanghai, China)

Table S2 Primer sequences for quantitative reverse transcription qRT-PCR of miniature pig samples

Gene	Primer sequence*
Pig *18S*	F: GCCCTATCAACTTTCGATGGTAGTC R: CCTTGGATGTGGTAGCCGTTTCTCA
Pig *iNOS*	F: AGGCTCAAATCGCAGCAGAATC R: TGCCCTCACAGCAGAGTTCCA
Pig *ARG1*	F: TTCTCCAAGGGTCAGCCACG R: GTCATTAGGGACATCAGCAAAGCA
Pig *FIZZ-1*	F: CCCTCCTCTTCCTCCCAACCCT R: TGGCGACATCCCGGATCTTC
Pig *MHC-II*	F: GAGCCCACGGTGACGGTGTA R: GTCCAGTCTCCATTAGGGATCAGG
Pig *IL-6*	F: GCCTTCAGTCCAGTCGCCTTCT R: GTGGCATCACCTTTGGCATCTTC
Pig *IFN-γ*	F: AGCTCTGGGAAACTGAATGACTTCG R: ACTGATGGCTTTGCGCTGGA
Pig *COL1A1*	F: GCGGAGGAGGCTATGACTTTGG R: GGGTCTGAGTGAAGGCTGTGAG
Pig *COLl3A1*	F: GAGTGGAAGAGCGGAGACTACTG R: GTCTCCATGTTGCAGAAGACTTTCA
Pig *CD68*	F: CCAAGCTCTACTGGGGCTCTTGG R: GACCTTGGTTTTGTTCGGGTTCA
Pig *CD163*	F: GGCCGAGTTAATGCCAGTGAGG R: GTCACGCCAGCGTCTTCTTTGT

*Designed and synthesized by Sangon Biotech (Shanghai, China)