

Supplementary Materials

Nanodefensin-encased hydrogel with dual bactericidal and pro-regenerative functions for advanced wound therapy

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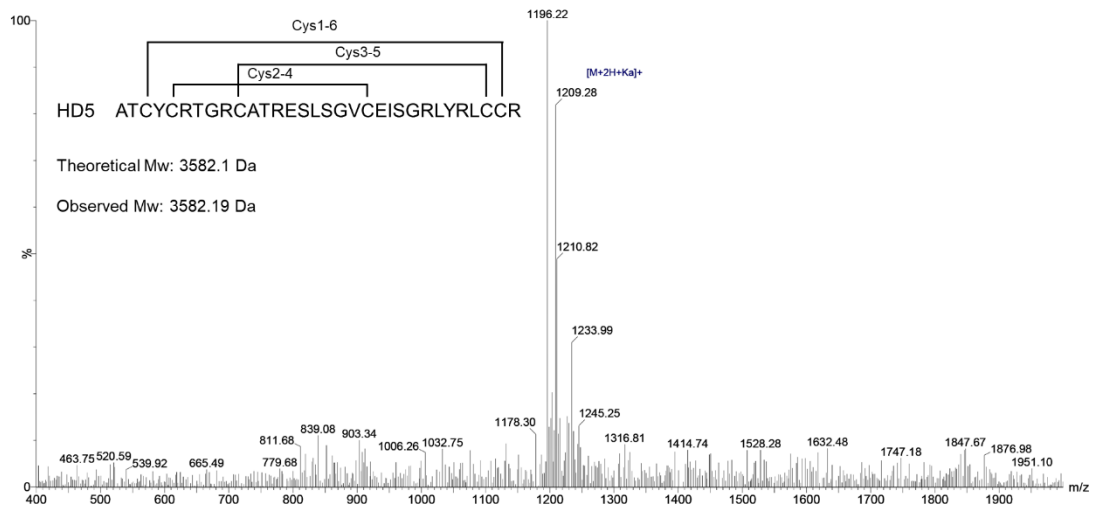


Figure S1. Electrospray ionization mass (ESI-MS) analysis of HD5. The mass-to-charge (m/z) is $[M+2H]/3$. Therefore the observed molecular weight is 3582.19 Da, in compared to the theoretical molecular weight of 3582.1 Da.

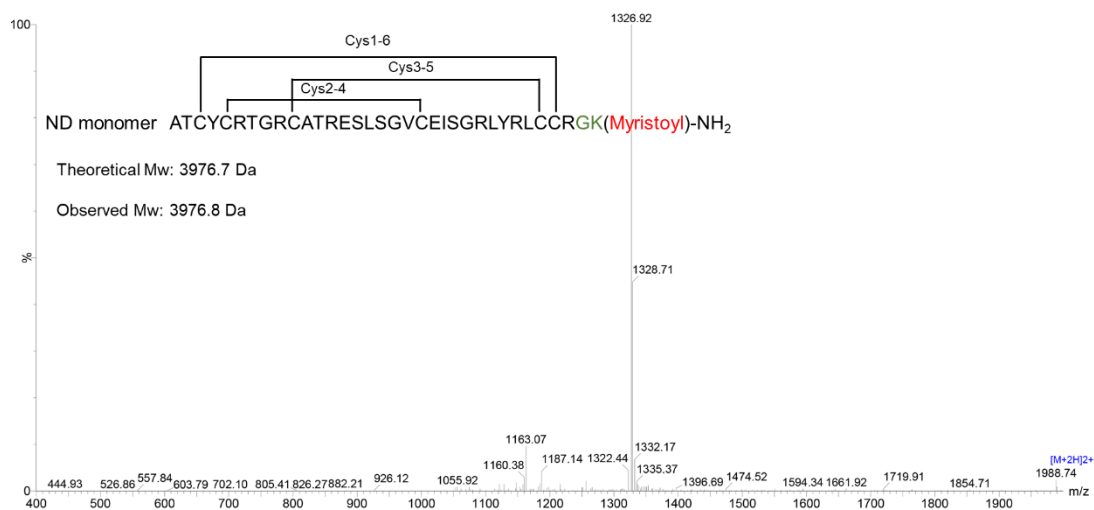


Figure S2. ESI-MS analysis of myristoylated HD5 (ND monomer). The mass-to-charge (m/z) is $[M+H]/3$. Therefore, the observed molecular weight is 3976.8 Da, in compared to the theoretical molecular weight of 3976.7 Da.

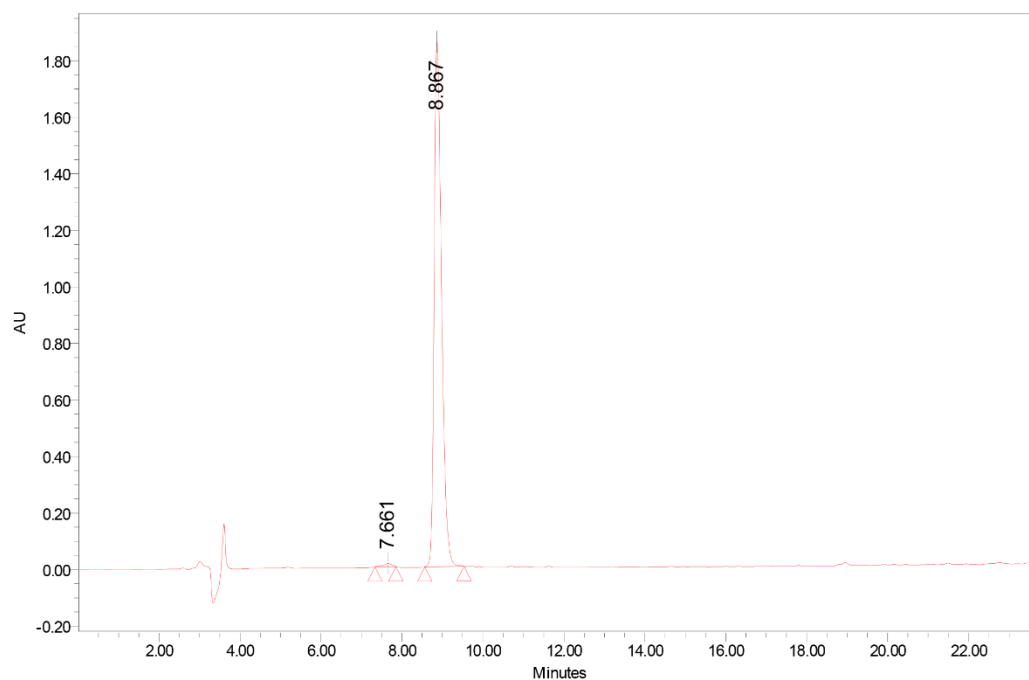


Figure S3. Representative chromatogram of HD5 analyzed by reverse-phase (RP) high-performance liquid chromatography (HPLC). The numbers indicate the retention times of HD5 (8.867 min) and the impurity (7.661 min).

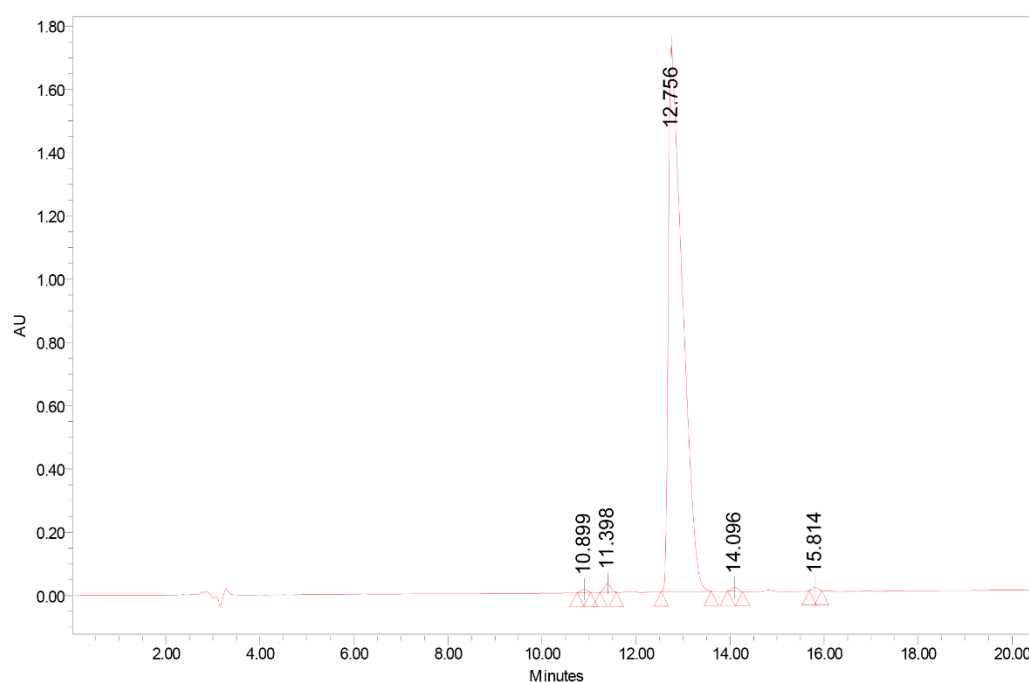


Figure S4. Representative chromatogram of ND monomer analyzed by HPLC. The numbers indicate the retention times of ND monomer (12.756 min) and several impurities (10.899, 11.398, 14.096, and 15.814 min).

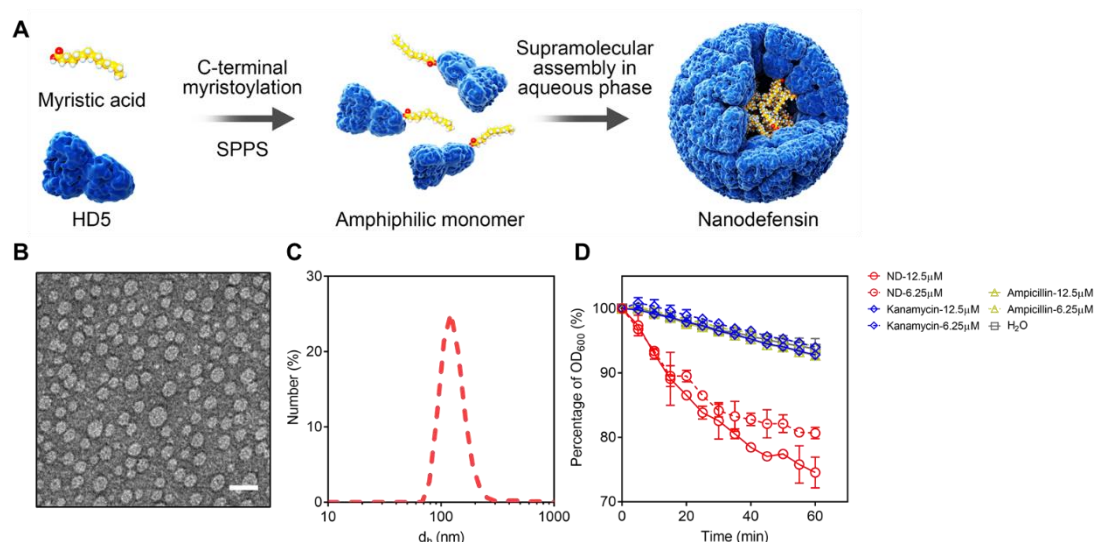


Figure S5. (A) Schematic diagram illustrating the molecular reprogramming and supramolecular assembly of ND. (B) Representative TEM image of the supramolecular nanostructures of ND in water. Scale bars, 100 nm. (C) Number distribution of hydrodynamic diameter (d_h) for ND. (D) Antimicrobial activity of ND against MRSA compared with the commercial antibiotics kanamycin and ampicillin. The bacterial suspension ($OD_{600} \approx 0.4$) was incubated with ND (6.25 μ M or 12.5 μ M), kanamycin (6.25 μ M or 12.5 μ M), ampicillin (6.25 μ M or 12.5 μ M), or sterile water and the OD_{600} was dynamically monitored every 5 min for 1 h. $n = 2$ per time point per group.

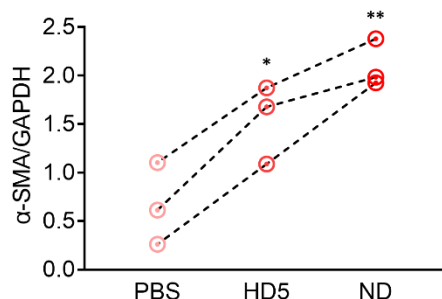


Figure S6. Quantification of expression levels of α -SMA in fibroblast 3T3 cells. Cells were stimulated by PBS, HD5 (12.5 μ g/mL), or ND (12.5 μ g/mL). Values represent the mean $\pm$ SD of three biological replicates ($n = 3$). GAPDH was adopted as an internal reference. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

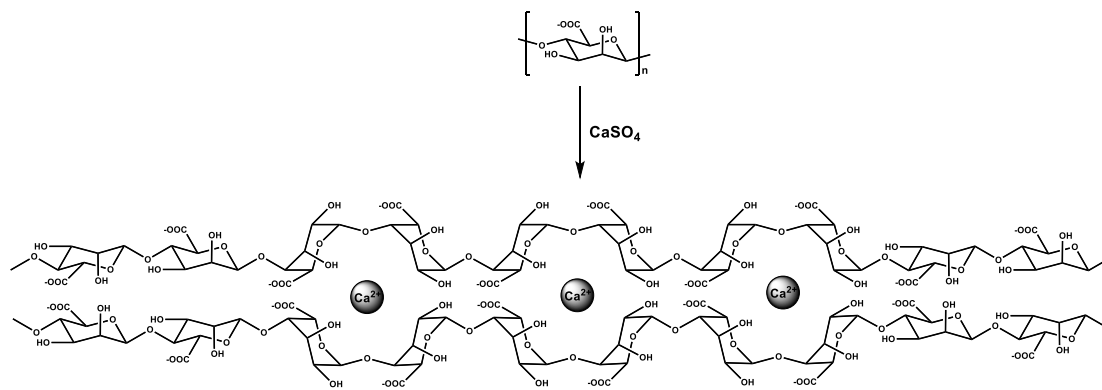


Figure S7. The chemical mechanism of ionic gelation of alginate hydrogel.

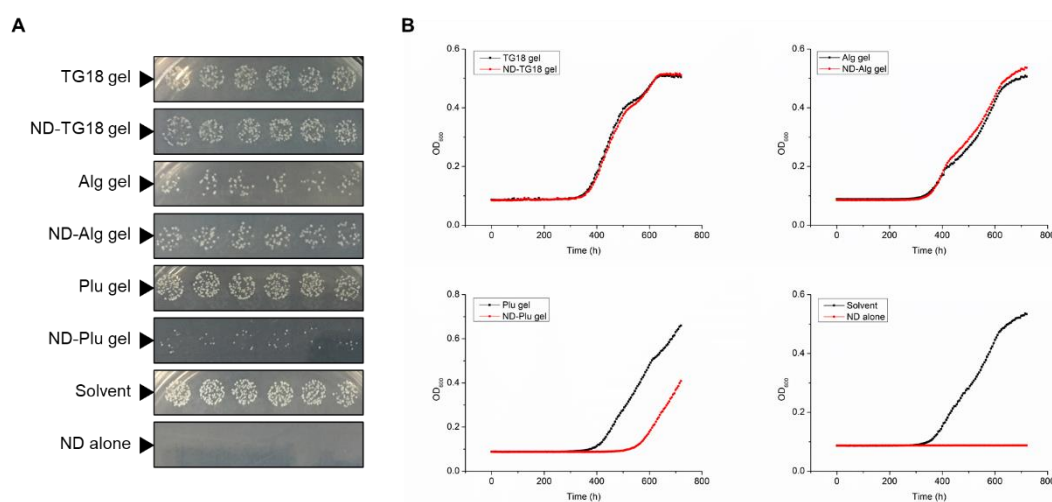


Figure S8. (A) Photographic images of viable count measurements of living MRSA after fully mixing with various hydrogel formulations. (B) The growth curves of MRSA suspension after fully mixing with various hydrogel formulations.

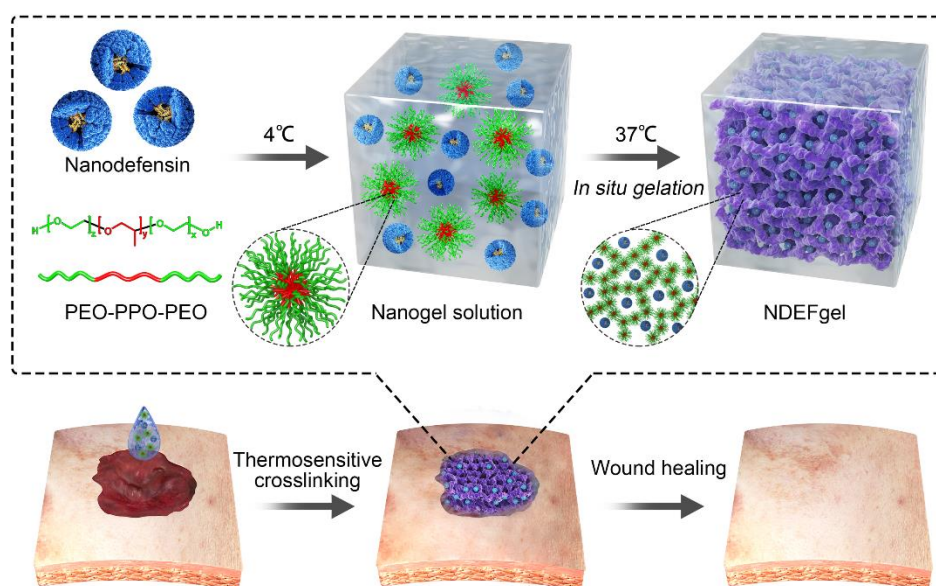


Figure S9. Schematic diagram illustrating that ND delivered by a hydrogel platform based on Plu effectively assists wound regeneration.

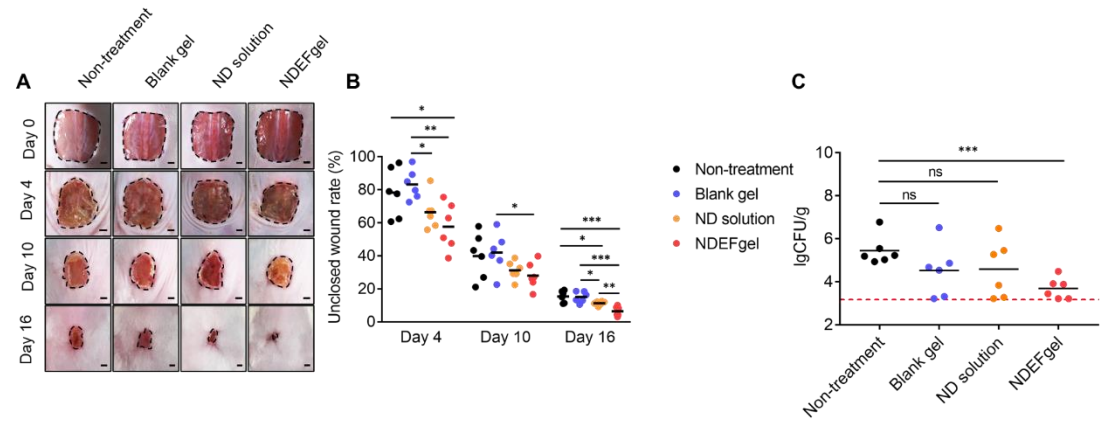


Figure S10. Therapeutic efficacy of NDEFgel on murine wound models complicated with MRSA infection, compared with that of non-treatment, blank gel, and ND solution. (A) Macroscopic imaging of wounds immediately following surgery (Day 0) and after 4, 10, or 16 days of treatment with different interventions including non-treatment, blank gel, ND solution, and NDEFgel. Scale bars, 2 mm. (B) Quantification of unclosed wound rates in the murine wound models after 16 days of treatment. $n = 6$ per group. (C) Determination of cutaneous bacterial burden in murine wound models treated with different formulations. Dashed line indicates the limit of detection. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; $****P < 0.0001$; ns, not significant.

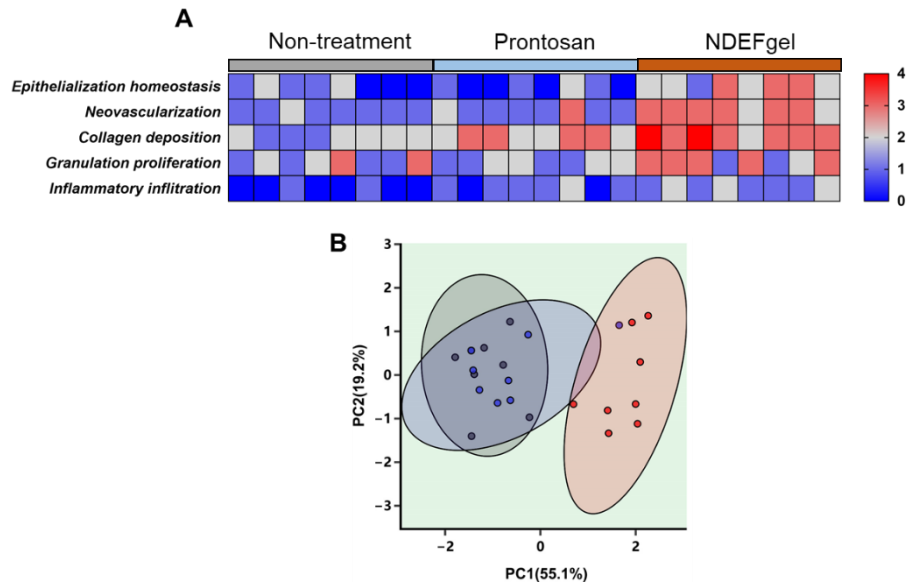
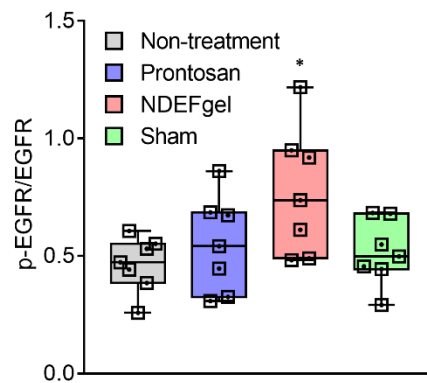
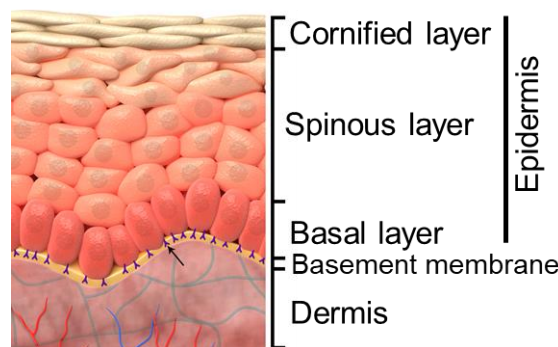


Figure S11. (A) Histopathological scores of skin tissues from murine wound models according to results of H&E staining and Masson's trichrome staining to evaluate different aspects of wound healing including epithelialization homeostasis, neovascularization, collagen deposition, granulation proliferation, and inflammatory infiltration. $n = 8$ per group. (B) Principal component analysis (PCA) of skin scores

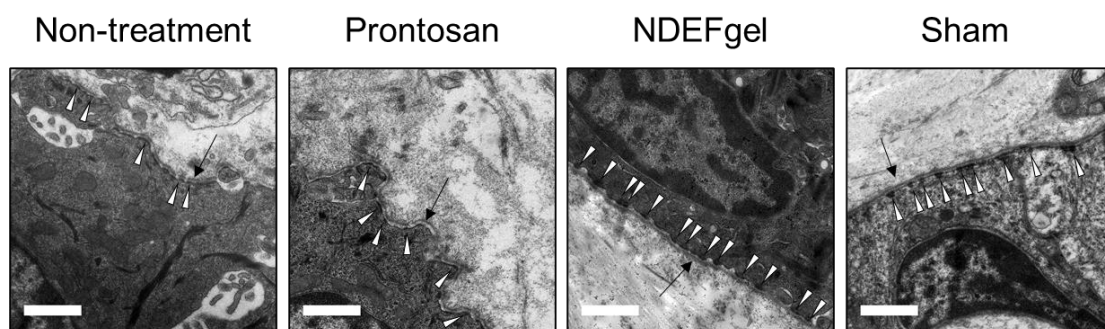
1 demonstrated that the cluster of the NDEFgel treatment group (red region) was
 2 separated from the clusters of all other groups, and that overlap was seen between
 3 Prontosan (blue region) and the non-treatment groups (grey region).
 4



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 6 **Figure S12. Protein levels of p-EGFR and EGFR in skin tissues.** The ratios of p-
 7 EGFR/EGFR were analyzed for different conditions including Non-treatment,
 8 Prontosan, NDEFgel, and Sham-operated treatment. Data are shown as means $\pm$ SD of
 9 seven biological replicates ($n = 7$). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.
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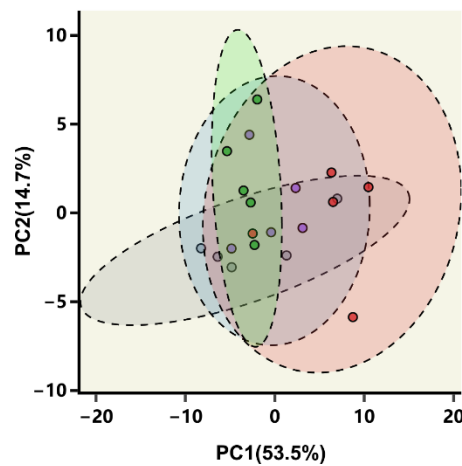


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 12 **Figure S13.** Schematic diagram illustrating the cutaneous structure.
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14
 15 **Figure S14. TEM characterization of skin tissues.** The morphology of non-treated
 16 skin tissues or treated with Prontosan, NDEFgel, and Sham-operation. Black arrows
 17 indicate the basement membrane of skin tissues. White arrows indicate the
 18 arrangements of hemidesmosomes attached to the basement membrane. Scale bars is 1
 19 μm .

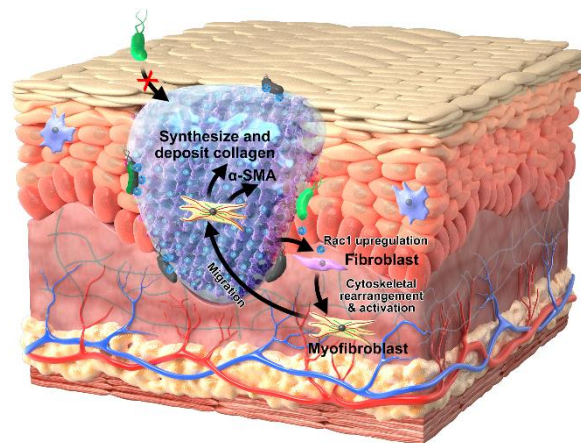
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3 **Figure S15.** PCA of the transcriptome in the wound microenvironment demonstrated
4 that the cluster of the NDEFgel treatment group was the most clearly segregated from
5 the other groups. The red region, NDEFgel group; blue region, Prontosan group; grey
6 region, non-treatment group; green region, sham-operated group.

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9 **Figure S16. Schematic diagram illustrating the possible mechanism of NDEFgel**
10 **effective management of wound regeneration.** 3D diagram of the epidermis can be
11 divided into three parts from top to bottom: the cornified region (yellow), the spinous
12 (orange) and basal (red) layers. The subcutaneous layer with adipose cells, blood
13 vessels and muscles cells were also depicted. NDEFgel (semi-transparent globular
14 structure) releases ND monomer (blue balls) that upregulates Rac1 in fibroblast cells
15 (pink). Besides, fibroblasts, highly heterogeneous cells, generally transform into α -
16 SMA-positive myofibroblasts to accelerate collagen deposition and wound closure. ND
17 monomer as an antimicrobial polypeptide attaches to the bacterial (green) membrane to
18 exhibit bactericidal activity.

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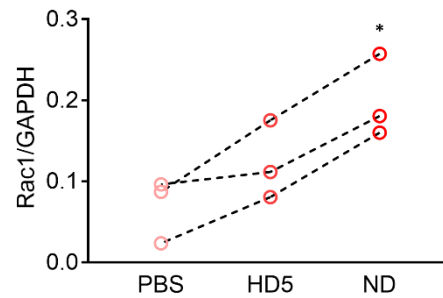


Figure S17. Quantification of expression levels of Rac1. Fibroblast 3T3 cells after stimulated by PBS, HD5 (12.5 $\mu\text{g/mL}$) or ND (12.5 $\mu\text{g/mL}$) were assayed with Rac1 expression. GAPDH was adopted as internal reference. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

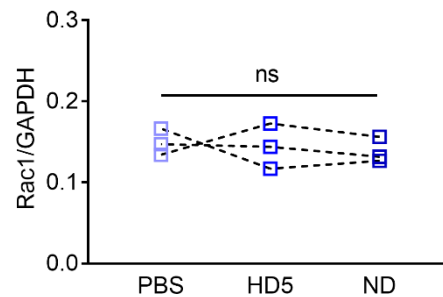


Figure S18. Quantification of expression levels of Rac1 in the presence of NSC23766. Fibroblast 3T3 cells after stimulated by PBS, HD5 (12.5 $\mu\text{g/mL}$) or ND (12.5 $\mu\text{g/mL}$) with pre-treatment of NSC23766 (50 μM), a selective inhibitor of Rac1, were assayed with Rac1 expression. GAPDH was adopted as internal reference. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

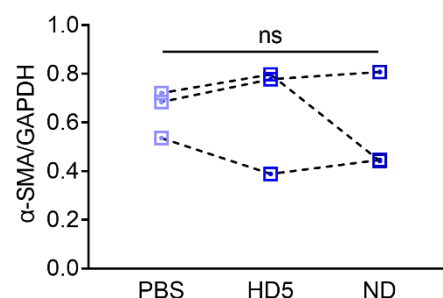


Figure S19. Quantification of expression levels of α -SMA in the presence of NSC23766. Fibroblast 3T3 cells after stimulated by PBS, HD5 (12.5 $\mu\text{g/mL}$) or ND (12.5 $\mu\text{g/mL}$) with pre-treatment of NSC23766 (50 μM), a selective inhibitor of Rac1, were assayed with α -SMA expression. GAPDH was adopted as an internal reference. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

1 **Table S1.** Mouse primers used for gene expression quantification.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
<i>HGF</i>	ATGTGGGGGACCAAACCTTCTG	GGATGGCGACATGAAGCAG
<i>FGF7</i>	CTCTACAGGTCATGCTTCCACC	ACAGAACAGTCTTCTCACCT
<i>FGF10</i>	TTTGGTGTCTTCGTTCCCTGT	TAGCTCCGCACATGCCTTC
<i>HB-EGF</i>	CGGGGAGTGCAGATACCTG	TTCTCCACTGGTAGAGTCAGC
<i>TGF-α</i>	CACTCTGGGTACGTGGGTG	CACAGGTGATAATGAGGACAGC
<i>TGF-β1</i>	ACGCCTGAGTGGCTGTCTTTTGAC	GGGCTGATCCCGTTGATTTCCACG
<i>VEGFA</i>	CGACACGGGAGACAATGGGATGAA	AGGGGCGGGGTGCTTTTGTAGACT
<i>FGF2</i>	GCGACCCACACGTCAAACCTA	TCCCTTGATAGACACAACCTCCTC
<i>MMP-2</i>	CTTCGCTCGTTTCCTTCAAC	ATGTCAGACAACCCGAGTCC
<i>MMP-9</i>	ATCCCCAGAGCGTCATTGCGG	CACGTAGCCACGTCGTCCAC
<i>PDGF-α</i>	CGCTGCACTGGCTGTTGTAA	GCCGGCTCATCTCCACCTCA
<i>PDGF-C</i>	GGAACAGAACGGAGTGCAAGA	TGAGGAAACTTCGGGCTGTG
<i>IGF-1</i>	GCCCAAGACTCAGAAGTCCCCG	TGCAGGTTGCTCAAGCAGCA
<i>Col1a1</i>	ACGTCCTGGTGAAGTTGGTC	TCCAGCAATACCCTGAGGTC
<i>Col3a1</i>	CTGGTCCTGTTGGTCCATCT	ACCTTTGTCACCTCGTGGAC
<i>Col12a1</i>	GCTATCCAGGTTCCGGCTAA	CCCTCCTGTATGATGCCGAC
<i>IL-1α</i>	TTGGTTAAATGACCTGCAACA	GAGCGCTCACGAACAGTTG
<i>IL-1β</i>	TTGACGGACCCCAAAAGAT	GAAGCTGGATGCTCTCATCTG
<i>IL-6</i>	ACCGCTATGAAGTTCCTCTCTGCAA	TGCAGGTTGCTCAAGCAGCA
<i>MCP-1</i>	CAACTCTCACTGAAGCCA	CAGCACAGACCTCTCTCTT
<i>TNF-α</i>	CTGAACCTTCGGGGTGATCGG	GGCTTGTCACCTCGAATTTGAGA