

Supporting Information

Biological Mechanism of Hydrolyzed Sponge-Mediated Stratum Corneum Penetration and Its Transdermal Permeation-Enhancing Efficacy:In Vitro and In Vivo Study

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S1. Raw uncropped Western blot images

Figure S1 shows the original, uncropped Western blot membrane images corresponding to **Figure 4** in the main manuscript. Each membrane is shown with molecular weight markers and full lane layout. All membranes were probed sequentially after stripping; full scans with visible Ponceau S staining are included. Original blots are labeled with experiment date (2024-05-15) and sample IDs.

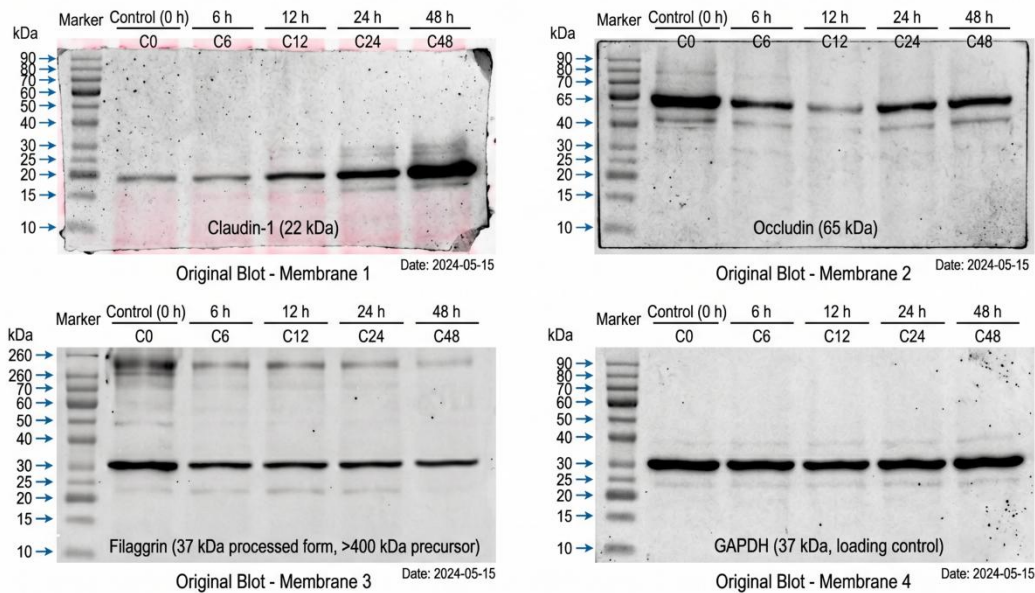


Figure S1. Raw uncropped Western blot images of (a) Claudin-1, (b) Occludin, (c) Filaggrin, and (d) GAPDH. Lanes: Marker, Control (0 h), 6 h, 12 h, 24 h, 48 h.

- (a) **Claudin-1 (22 kDa)**: Full membrane visible with background noise, no cropping.
- (b) **Occludin (65 kDa)**: Gradual decrease then recovery across time points.
- (c) **Filaggrin (37 kDa processed form, >400 kDa precursor)**: No visible change.
- (d) **GAPDH (37 kDa, loading control)**: Consistent bands across all lanes.

The original uncropped scans are provided as high-resolution TIFF files in the accompanying archive (see S5).

S2. Franz diffusion cell time- concentration profiles

Cumulative permeation values ($\mu\text{g}/\text{cm}^2$) at each time point for four drugs (lidocaine HCl, fluorescein sodium, FITC-BSA, and protoporphyrin IX) are presented below. Data are shown as mean $\pm$ SD, $n = 6$ per group. Control = untreated skin; SHS = treated with SHS; Silicon microneedle = treated with silicon microneedles.

Table S1. Cumulative permeation of lidocaine HCl.

Time (h)	Control ($\mu\text{g}/\text{cm}^2$)	SHS ($\mu\text{g}/\text{cm}^2$)	Silicon microneedle ($\mu\text{g}/\text{cm}^2$)
0.5	5.2 ± 1.1	32.1 ± 4.5	25.3 ± 3.8
1	12.4 ± 2.3	78.5 ± 9.2	58.7 ± 7.1

Time (h)	Control ($\mu\text{g}/\text{cm}^2$)	SHS ($\mu\text{g}/\text{cm}^2$)	Silicon microneedle ($\mu\text{g}/\text{cm}^2$)
2	25.8 ± 4.2	168.3 ± 18.4	125.4 ± 14.2
4	48.3 ± 6.5	322.5 ± 31.2	241.6 ± 26.5
6	70.2 ± 8.9	475.2 ± 44.3	361.8 ± 35.7
8	92.5 ± 11.3	598.4 ± 56.7	468.2 ± 45.2
12	125.3 ± 15.2	760.3 ± 72.4	612.5 ± 58.3
24	158.6 ± 21.3	920.5 ± 86.4	745.3 ± 67.2

Table S2. Cumulative permeation of fluorescein sodium.

Time (h)	Control ($\mu\text{g}/\text{cm}^2$)	SHS ($\mu\text{g}/\text{cm}^2$)	Silicon microneedle ($\mu\text{g}/\text{cm}^2$)
0.5	1.2 ± 0.3	15.4 ± 2.1	10.2 ± 1.8
1	2.8 ± 0.5	38.7 ± 4.5	25.6 ± 3.2
2	5.9 ± 0.9	82.3 ± 8.7	55.3 ± 6.1
4	10.4 ± 1.6	158.4 ± 16.2	108.7 ± 11.5
6	15.8 ± 2.3	235.6 ± 22.5	162.4 ± 17.3
8	22.1 ± 3.1	312.8 ± 30.1	218.6 ± 22.8
12	32.5 ± 4.8	385.3 ± 37.4	285.3 ± 29.5
24	47.1 ± 8.3	432.6 ± 41.5	339.5 ± 32.7

Table S3. Cumulative permeation of FITC- BSA (quantified by BCA).

Time (h)	Control ($\mu\text{g}/\text{cm}^2$)	SHS ($\mu\text{g}/\text{cm}^2$)	Silicon microneedle ($\mu\text{g}/\text{cm}^2$)
0.5	0.1 ± 0.05	0.8 ± 0.2	0.5 ± 0.1
1	0.3 ± 0.1	2.1 ± 0.4	1.3 ± 0.3
2	0.6 ± 0.2	4.5 ± 0.8	2.8 ± 0.6
4	1.0 ± 0.3	8.2 ± 1.1	5.2 ± 0.9
6	1.5 ± 0.4	11.3 ± 1.9	7.4 ± 1.2
8	2.0 ± 0.6	14.2 ± 2.3	9.3 ± 1.5
12	2.6 ± 0.8	17.1 ± 2.8	11.2 ± 1.9
24	3.2 ± 1.1	19.5 ± 3.7	12.8 ± 2.9

Table S4. Cumulative permeation of protoporphyrin IX (PpIX).

Time (h)	Control ($\mu\text{g}/\text{cm}^2$)	SHS ($\mu\text{g}/\text{cm}^2$)	Silicon microneedle ($\mu\text{g}/\text{cm}^2$)
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Time (h)	Control ($\mu\text{g}/\text{cm}^2$)	SHS ($\mu\text{g}/\text{cm}^2$)	Silicon microneedle ($\mu\text{g}/\text{cm}^2$)
0.5	0.3 ± 0.1	5.2 ± 0.8	3.1 ± 0.5
1	0.8 ± 0.2	14.6 ± 1.9	9.4 ± 1.2
2	1.5 ± 0.4	28.3 ± 3.2	18.7 ± 2.4
4	2.6 ± 0.6	48.2 ± 5.1	32.5 ± 3.8
6	3.8 ± 0.9	62.5 ± 6.7	44.2 ± 4.9
8	5.0 ± 1.1	73.4 ± 7.8	53.1 ± 5.7
12	6.2 ± 1.4	81.3 ± 8.5	63.8 ± 6.8
24	7.0 ± 1.8	86.4 ± 9.2	71.2 ± 7.4

Raw Excel files containing all individual replicates, standard curves, and chromatograms are provided in the archive (S5).

S3. Transepidermal water loss (TEWL) raw data

TEWL ($\text{g}/\text{m}^2/\text{h}$) was measured on days 0, 1, 2, 3, 5, and 7 after cessation of SHS treatment. $n = 6$ mice (SHS group). Day 0 = baseline before SHS treatment; days 1–7 = post-intervention cessation (after 3 consecutive days of SHS application).

Table S5. Individual mouse TEWL values ($\text{g}/\text{m}^2/\text{h}$).

Mouse ID	Day 0 (baseline)	Day 1	Day 2	Day 3	Day 5	Day 7
M1	8.2	16.8	11.6	9.4	8.5	8.1
M2	9.1	17.2	12.1	9.8	8.9	8.4
M3	7.8	15.9	10.9	8.7	7.9	7.6
M4	8.5	16.4	11.4	9.2	8.3	8.0
M5	8.0	15.5	10.5	8.5	7.8	7.5
M6	8.2	17.0	11.8	9.5	8.6	8.2
Mean	8.3	16.5	11.4	9.2	8.3	8.0
SD	1.2	2.1	1.8	1.5	1.3	1.1

Raw time-stamped device outputs are included in the archive (S5).

S4. ARRIVE 2.0 checklist

The completed ARRIVE 2.0 Essential 10 checklist is provided in **Table S6**. The full PDF including the Recommended Set (items 11–20) is included in the archive (S5).

Table S6. ARRIVE 2.0 Essential 10 checklist.

Item	Description	Where in manuscript	Section / Page
1	Study design	2.3, 2.6	In vitro and in vivo experiments, groups, sample sizes
2	Sample size	2.3 (n=6), 2.6 (n=6 per group)	Materials and Methods
3	Inclusion and exclusion criteria	Not applicable (no exclusions)	—
4	Randomisation	2.6 (randomly divided into 3 groups)	Materials and Methods
5	Blinding	Not explicitly stated – suggested addition: "Outcome assessment was blinded"	(Suggested addition)
6	Outcome measures	2.3 (cumulative permeation, Jss), 2.5 (protein expression), 2.6 (TEWL, fluorescence)	Materials and Methods
7	Statistical methods	2.7 (ANOVA, t- test, P<0.05)	Materials and Methods
8	Experimental animals	2.3 (SD rats), 2.6 (Balb/c mice), strain, sex, weight, age	Materials and Methods
9	Experimental procedures	2.2–2.6 (detailed)	Materials and Methods
10	Results	3.1–3.5 (all quantitative data with SD and P values)	Results

Recommended Set (items 11–20) is provided in the separate PDF file (see S5).

S5. Raw data archive contents (ZIP file)

A compressed archive ([Raw_Data.zip](#), approx. 450 MB) contains all raw data files supporting the manuscript. The contents are listed below.

File name	Description	Format
SEM_images_original	Unprocessed SEM micrographs of SHS (Figure 1A) at 500×, 1000×, 2000×	.tiff
Confocal_FITC_BSA	Raw LSM files and exported images for FITC- BSA skin distribution (Figures 3C, 5A)	.lsm, .tiff
Western_blot_original_scans	High-resolution scans of all uncropped membranes (Claudin- 1, Occludin, Filaggrin, GAPDH) with visible markers	.tiff
Western_blot_densitometry_raw	Original ImageJ measurement values for each band	.xlsx
HPLC_chromatograms_raw	Raw chromatogram files for lidocaine, fluorescein sodium, PpIX quantification	.txt (exported)
BCA_BSA_raw_absorbance	Raw 562 nm absorbance readings for BSA standard curve and samples	.xlsx
TEWL_raw_individual	Individual mouse TEWL values (same as Table S5) plus time-stamped raw device outputs	.xlsx
Histology_images_raw	HE and Nile Red staining full- slide scans (Figures 3A, 3B)	.tiff
ARRIVE_checklist.pdf	Completed ARRIVE 2.0 checklist (Essential 10 + Recommended Set) with tick- boxes and page references	.pdf

End of Supporting Information