NOTE: Please save this file locally before filling in the table, DO NOT work on the file within your internet browser as changes will not be saved. Adobe Acrobat Reader (available free here) is recommended for completion.

The ARRIVE guidelines 2.0: author checklist

The ARRIVE Essential 10

These items are the basic minimum to include in a manuscript. Without this information, readers and reviewers cannot assess the reliability of the findings.

	Item	Recommendation	Section/line number, or reason for not reporting
Study design	1	For each experiment, provide brief details of study design including: a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated. b. The experimental unit (e.g. a single animal, litter, or cage of animals).	n vitro transdermal experiment (Methods 2.3): three groups – blank control (drug solution alone), SHS group (drug + 10 mg/mL SHS), silicon microneedle group (microneedle pre-treatment + drug). In vivo mouse experiment (Methods 2.6): same three groups. Skin irritation test (Methods 2.2): left side SHS suspension, right side saline control (within-animal control). Western blot (Methods 2.5): time-course with 0 h as baseline control. In vitro Franz cell: each diffusion cell with a skin disc = one experimental unit (n=6 per group). In vivo mouse: each individual mouse (n=6 per group). Western blot: each skin sample from one mouse (n=4 per time point). Rabbit irritation test: each rabbit served as its own control (n=3).
Sample size	2	a. Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used. b. Explain how the sample size was decided. Provide details of any *a priori* sample size calculation, if done.	In vitro (Franz cell): n=6 per group (blank, SHS, silicon microneedle) for each of 4 drugs → 6 × 3 × 4 = 72 diffusion cells. In vivo mouse (FITC-BSA): n=6 per group → total 18 Balb/c mice. Skin barrier recovery (TEWL): n=6 mice (SHS group only). Western blot: n=4 per time point (0,6,12,24,48 h) → 20 mice (additional). Cytotoxicity assay: triplicate wells per concentration (0.1,0.5,1.0,5.0 mg/mL) at 3 time points – not animal. Rabbit irritation test: n=3 New Zealand white rabbits. Total animals: ≈ 3 rabbits + 18 + 20 +? SD rats (skin donors) = approximately 50–60 animals. Exact total not explicitly summed in manuscript. Not reported. The manuscript does not describe any a priori sample size calculation or power analysis. It only states “n=6”, “n=4” etc. without justification.
Inclusion and exclusion criteria	3	a. Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established *a priori*. If no criteria were set, state this explicitly. b. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so. c. For each analysis, report the exact value of *n* in each experimental group.	No explicit inclusion/exclusion criteria described. Animals were selected based on standard specifications: healthy male SD rats (200-250 g), female Balb/c mice (6-8 weeks, 18-22 g), male New Zealand rabbits (2.5-3.0 kg). No criteria for excluding animals during the experiment were stated. No exclusions reported. The manuscript implicitly assumes all animals and samples were included. Reported: In vitro – “n=6” for each group (Figure 2 legend). Western blot – “n=4” (Figure 4 legend). In vivo TEWL – “n=6” (Figure 5 legend). Rabbit irritation – “n=3” (Methods 2.2).
Randomisation	4	a. State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence. b. Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly.	For in vivo mouse experiment: “18 Balb/c mice were randomly divided into 3 groups” (Methods 2.6) – randomisation stated but method (e.g., random number table, software) not provided. For in vitro and rabbit experiments: no mention of randomisation. Not described. No information on whether order of treatments, measurement sequence, or cage location was controlled.
Blinding	5	Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).	Not reported. The manuscript does not mention blinding at any stage (allocation, experiment conduct, outcome assessment, or data analysis).

Outcome measures	6	a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes). b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.	In vitro: cumulative permeation (μg/cm²), steady-state transdermal rate (Jss), permeation enhancement factor (ER). In vivo (mouse): fluorescence distribution and intensity (FITC-BSA), transepidermal water loss (TEWL, g/m²/h). Histology: stratum corneum integrity (HE staining), lipid organisation (Nile Red). Molecular: protein expression of Claudin-1, Occludin, Filaggrin (Western blot). Safety: relative cell proliferation (MTT), skin irritation score, intradermal reaction. Not specified. The study does not explicitly declare a primary outcome measure. Cumulative permeation (in vitro) and TEWL (in vivo) appear to be key, but no primary outcome is stated.
Statistical methods	7	a. Provide details of the statistical methods used for each analysis, including software used. b. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met.	Methods 2.7: "Data analysis was performed using SPSS 22.0 software. Measurement data were expressed as Mean ± SD. One-way ANOVA was used for comparisons among multiple groups, and independent samples t-test was used for comparisons between two groups. A P-value <0.05 was considered statistically significant." Not reported. No mention of testing for normality, homogeneity of variance, or other assumptions.
Experimental animals	8	a. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight. b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.	Rats: "healthy male SD rats (200-250 g)" (Methods 2.3). Mice: "Balb/c mice (female, 6-8 weeks old, body weight 18-22 g)" (Methods 2.6). Rabbits: "New Zealand white rabbits (male, 2.5-3.0 kg)" (Methods 2.2). Provenance: Rats from "Silek Jingda" (Methods 2.3); mice and rabbits – source not stated. Health/immune status: Mice housed under SPF conditions (Methods 2.6). No genetic modifications. No previous procedures reported.
Experimental procedures	9	For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: a. What was done, how it was done and what was used. b. When and how often. c. Where (including detail of any acclimatisation periods). d. Why (provide rationale for procedures).	All procedures are described in sufficient detail: • SHS characterisation (SEM, cytotoxicity, irritation tests) – Methods 2.2. • In vitro transdermal (Franz cell) – Methods 2.3: 32 °C, 300 rpm, sampling at 0.5,1,2,4,6,8,12,24 h, rationale for drug selection. • Histology and confocal – Methods 2.4: 30 min treatment, fixation, staining. • Western blot – Methods 2.5: time points 0-48 h, antibody dilutions. • In vivo mouse – Methods 2.6: 2 h after administration, euthanasia. • TEWL recovery – days 0,1,2,3,5,7 post-intervention, with 30 min acclimatisation. • Rationale implicitly provided for each (e.g., to mimic clinical use, to assess reversibility)
Results	10	For each experiment conducted, including independent replications, report: a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range). b. If applicable, the effect size with a confidence interval.	All results include mean ± SD – see Figures 2, 4, 5 and Tables 1, 2, 3. Examples: lidocaine cumulative permeation "920.5±86.4 μg/cm²" (Fig. 2A); TEWL "8.3±1.2 g/m²/h" baseline (Fig. 5B); protein expression "42.3±8.7%" (Fig. 4). Not reported. The manuscript reports P values and fold changes (e.g., "5.8-fold", "12.3-fold") but does not provide effect sizes (e.g., Cohen's d) or confidence intervals around the effect estimates.

The Recommended Set

These items complement the Essential 10 and add important context to the study. Reporting the items in both sets represents best practice.

		Item Recommendation	Section/line number, or reason for not reporting
Abstract	11	Provide an accurate summary of the research objectives, animal species, strain and sex, key methods, principal findings, and study conclusions.	Abstract (page 1 of manuscript). Species/strains/sex: SD rats (male, 200–250 g), Balb/c mice (female, 6–8 weeks, 18–22 g), New Zealand white rabbits (male, 2.5–3.0 kg). Key methods: SEM, Franz cell, Western blot, TEWL. Main findings: SHS increases permeation 5.8–12.3×, reversibly downregulates Claudin-1/Occludin
Background	12	a. Include sufficient scientific background to understand the rationale and context for the study, and explain the experimental approach. b. Explain how the animal species and model used address the scientific objectives and, where appropriate, the relevance to human biology.	Introduction (section 1). a. TDDS limitations, stratum corneum barrier, microneedle technology, sponge spicules as natural microneedles. b. Section 2.3 & 2.6: Rat skin (common ex vivo model for permeation), mouse skin (in vivo barrier recovery), rabbit skin (irritation test per GB/T16886). Relevance to human biology discussed in Discussion 4.5 (limitations: rat vs. human skin differences).
Objectives	13	Clearly describe the research question, research objectives and, where appropriate, specific hypotheses being tested.	Introduction (last paragraph): "This study systematically elucidates the biological mechanisms … from three aspects: lipid structure, intercellular junctions, and barrier protein expression." Hypotheses: SHS regulates skin barrier proteins; permeation kinetics differ by drug physicochemical properties.

Ethical statement	14	Provide the name of the ethical review committee or equivalent that has approved the use of animals in this study, and any relevant licence or protocol numbers (if applicable). If ethical approval was not sought or granted, provide a justification.	Declarations – Ethics approval and consent to participate (page after Discussion): "Xiamen University Laboratory Animal Ethics Committee (Approval No.: XMULAC-2024-015, approved 10 March 2024)." Also states compliance with ARRIVE guidelines and GB/T16886.
Housing and husbandry	15	Provide details of housing and husbandry conditions, including any environmental enrichment.	Section 2.6 (In vivo transdermal validation) : "Mice were housed under specific pathogen-free conditions at 22 ± 2 °C, 55 ± 5% humidity, a 12 h light/dark cycle, with free access to water and standard chow." No environmental enrichment reported for rats/rabbits – this is a limitation but not required for short-term ex vivo or acute irritation studies (stated in Methods 2.2 & 2.3).
Animal care and monitoring	16	a. Describe any interventions or steps taken in the experimental protocols to reduce pain, suffering and distress. b. Report any expected or unexpected adverse events. c. Describe the humane endpoints established for the study, the signs that were monitored and the frequency of monitoring. If the study did not have humane endpoints, state this.	a. Ethics statement: "Humane endpoints were rigorously applied throughout the study to minimize animal distress and suffering." No specific analgesics reported; however, the interventions (topical application, intradermal injection) are minimally invasive. b. Section 2.2 & Results 3.1: Only mild erythema (resolved within 24 h) and no intradermal irritation – no unexpected adverse events. c. No explicit humane endpoints listed beyond general statement. Reason: Study endpoints were fixed time points (e.g., euthanasia at 2 h post-administration, or after 24 h of skin observation). Animals were not allowed to suffer severe distress. The absence of detailed humane endpoints is noted as a limitation (but follows GB/T16886 guidelines for irritation testing).
Interpretation/ scientific implications	17	a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature. b. Comment on the study limitations including potential sources of bias, limitations of the animal model, and imprecision associated with the results.	a. Discussion 4.1–4.3: Dual physical-biological mechanism; comparison with literature (tight junction regulation, high-density microchannels). b. Discussion 4.5 (Research limitations): "In vitro transdermal experiment used rat skin, which differs from human skin in stratum corneum thickness and follicular density … short-term effect only … molecular mechanism of tight junction downregulation unclear." Also mentions potential bias (no blinding stated – though not required in standard permeation studies).
Generalisability/ translation	18	Comment on whether, and how, the findings of this study are likely to generalise to other species or experimental conditions, including any relevance to human biology (where appropriate).	Discussion 4.4 (Biosafety and clinical translation prospects) and 4.5: SHS as a natural microneedle with broad applicability; comparison with human skin limitations; potential for clinical translation (e.g., vaccine delivery, photodynamic therapy). Also notes that reversibility (48–72 h) suggests safe use in humans.
Protocol registration	19	Provide a statement indicating whether a protocol (including the research question, key design features, and analysis plan) was prepared before the study, and if and where this protocol was registered.	Not reported. The study did not pre-register a protocol. (Reason: This is an exploratory mechanistic study, not a clinical trial; many preclinical studies do not require registration.)
Data access	20	Provide a statement describing if and where study data are available.	Declarations – Availability of data and materials: "All raw data … included in this published article and its supplementary information files. A key dataset … deposited in Figshare (DOI:10.6084/m9.figshare.26345123). Core datasets … available from the corresponding author upon reasonable request." Also supplementary materials include raw Western blot images, Franz cell data, TEWL values, and ARRIVE checklist.
Declaration of interests	21	a. Declare any potential conflicts of interest, including financial and non-financial. If none exist, this should be stated. b. List all funding sources (including grant identifier) and the role of the funder(s) in the design, analysis and reporting of the study.	a. Declarations – Competing interests: "The authors declare no known competing financial interests or personal relationships … The funders had no role … The supplier of SHS … had no role." b. Declarations – Funding: National Natural Science Foundation of China (Grant No. 82273856), Key Project of Fujian Provincial Science and Technology Plan (2023Y0021), Xiamen Municipal Marine Development Special Fund (XMHC2022015). Role: "The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript."