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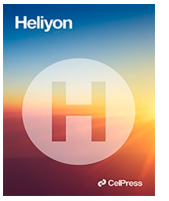

Research article

Study on skin infection model of *Staphylococcus aureus* based on analytic hierarchy process and Delphi method

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ABSTRACT

Purpose: Infectious skin diseases are a type of inflammatory skin lesions caused by pathogenic microorganisms. Because of the uncertainty of methodology, the skin infection model usually have low replication rate and lack of good evaluation system. We aimed to establish multi-index and comprehensive evaluation method for *Staphylococcus aureus* (*S.aureus*) skin-infection models through Analytic hierarchy process (AHP) and Delphi method, and screen high quality animal models through it.

Materials and methods: Firstly, the evaluation indicators of skin infection were collected basing on literature research. The weight of the evaluation indicators were decided according to AHP and Delphi method. Then different ulcer models (mouse or rat) infected by *S. aureus* were selected as the research objects.

Results: The evaluation indicators were classified into four groups of criteria (including ten sub-indicators) and given different weights, physical sign changes (0.0518), skin lesion appearance (0.2934), morphological observation (0.3184), etiological examination (0.3364). Through the evaluation system, we screened and found that the mouse ulcer model which caused by a round wound and 1.0×10^{10} CFU/mL (0.1 mL) bacterial concentration got the highest comprehensive score, and also found that the model which caused by a 1.5 cm-round wound and 1.0×10^{10} CFU/mL (0.2 mL) maybe the best rat ulcer model.

Conclusions: This study has established an evaluation system based on AHP and Delphi method, also provided the best skin ulcer models selected by this system, the models are suitable for disease research and drug development research of skin ulcer.

1. Introduction

Infectious skin diseases are a type of inflammatory skin lesions caused by pathogenic microorganisms, which are very commonly seen in clinical and seriously affects people's health [1]. Skin wound infection caused by *Staphylococcus aureus* (*S.aureus*), which is a

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critical clinical problem due to long hospitalization times, significant morbidity and mortality, as well as considerable medical resource consumption [2,3]. With the emergence of methicillin resistant *S. aureus* strains, current antibiotic treatments are becoming ineffective in combating *S. aureus* infection [4,5], and more new antimicrobial approaches are urgently needed. However, good animal models are the precondition of drug research. At present, there is little research on the skin microorganism infection models, so the replication success rate of the models and stability in modeling are not satisfactory, and there is still lack of a good model evaluation system in experimental methodology of dermatology [6,7].

A useful animal model system should be clinically relevant, experimentally robust, ethically acceptable, and convenient to perform and should provide reliable and reproducible results [8]. But the animal models of skin infection reported by different researchers varied in animal species and sex, microorganism strains, the number of microorganisms applied, the size of the wounds (depth and dimensions), and etc [9,10]. Moreover, these infection animal models vary significantly in the parameters, because there isn't uniform reference standard of comprehensive evaluation index for skin disease animal models, which usually judged by local appearance and pathological changes. Therefore, to establish comprehensive evaluation method for skin infection models and even other animal models of dermatosis, we first considered how to establish standard protocols for evaluation system, which could enable results from different research aspects to be compared.

Analytic hierarchy process (AHP) is a multiple attributes evaluation method developed by Dr. Saaty [11], which uses a hierarchy structure to systemize a complicated question and to divide the decision-making items into different levels. Furthermore, different levels are used to divide a question into different hierarchies so as to make a complicated bigger question into a smaller question, then AHP procedures are used to evaluate their relative importance and integrations respectively so that a final solution can be found out [12]. AHP has already been applied in many fields, such as medicine and medical diagnosis [13–15]. The Delphi method is a qualitative research approach used to gain consensus or set priorities through expert opinions on a real-world problem [16], which can make full use of expert knowledge, experience and wisdom to achieve the goal of group decision making [17–19]. The use of the Delphi increased substantially as the method became widely known in many professions, including nursing and medicine [20,21]. The combination of these two methods can be used to select the main evaluation indexes and provide a quantitative evaluation assessment to guide decision-making [17,22]. However, few reports have been addressed on the application of AHP and Delphi method on animal model. Therefore, AHP combined with Delphi method, could be a promising tool to assess and select experimental animal model for development of new drug efficacy evaluation, and to provide a scientific, normative and practical reference.

This paper took the skin wound infection model of *S. aureus* as an example, and presented a thorough description of AHP and Delphi method application in identifying parameter weights, which could be used for developing an evaluation system for animal model of

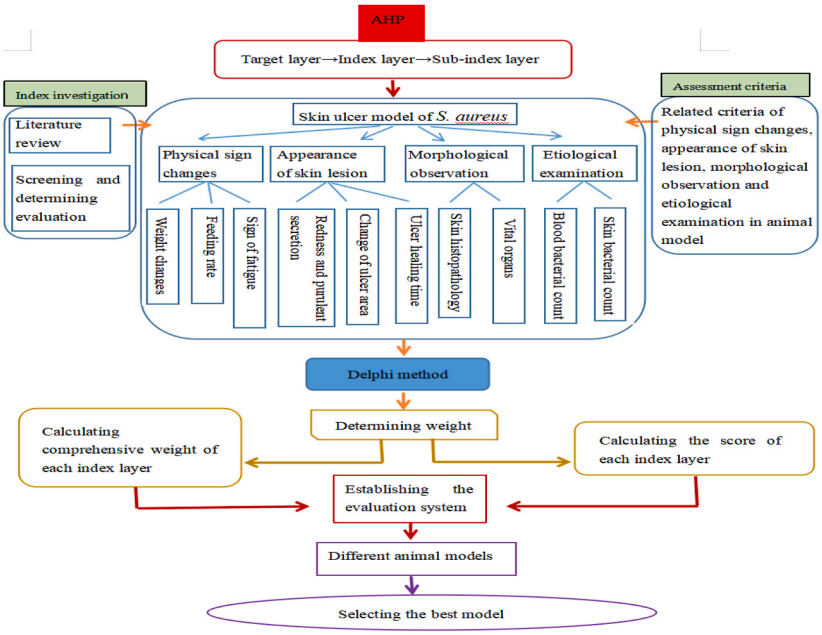

Fig. 1. Flow chart of establishing the evaluation system of skin wound infection animal model based on AHP and Delphi method. In order to build a hierarchical model, it is divided into target layer, index layer and sub-index layer. According to index investigation and assessment criteria, the evaluation index system was composed of four indexes: physical sign changes, appearance of skin lesion, morphological observation, and etiological examination. The sub-indicators of physical sign changes are weight changes, feeding rate, sign of fatigue. The sub-indicators of appearance of lesions are redness and purulent secretion of skin, change of ulcer area. The sub-indicators of morphological observation are skin histopathology and observation of vital organic index. The sub-indicators of etiological examination are blood and skin bacterial count. Then the comprehensive evaluation was constructed by AHP, combining with Delphi method to determine weight, and at last established the evaluation system of skin wound infection animal model, it was applied to different animal models to select the best model.

skin infection.

2. Materials and methods

2.1. Frame for evaluation system of skin wound infection animal model

A flow chart showed the research process as Fig. 1, the main aim of this study was to evaluate the animal models of skin wound infection and construct a comprehensive evaluation system of skin wound infection based on AHP and Delphi. The procedure is to build the analytic hierarchy structure, construct the judgment matrix to determine the weight of each index, and calculate the weight of each evaluation factor. Firstly, we need to identify the evaluation index system through literature screening [23–27]. Secondly, AHP was used to establish the hierarchical structure of evaluation system and identify each index level. Thirdly, in order to determine the weight of each index level and assessment criteria, we invited different experts from different fields, including pharmacological, biological, microbiological, zoological, and clinician experts. They were invited to answer the questionnaires to determine the weight of each index, and also offered assessment criteria to evaluating index. Lastly, samples of each indicator were collected from different models and analyzed to obtain the measurement data, after calculating the scores of each layer, we obtained the comprehensive evaluation results of different animal model, which could provide evidence for selecting the best model and establishing evaluation system of skin wound infection animal model.

2.2. Establishment of evaluation index system

First, a variety of skin infection models were collected through literature review, from this, we selected comprehensive indicators of skin wound infection model. Ultimately, 4 indicators were extracted and classified into physical sign changes, appearance of skin lesion, morphological observation, etiological examination, and they were further divided into 10 sub-indicators. All the selected indicators were presented in Fig. 1.

2.3. Application of Delphi method to weight the evaluation index

In order to determine the weight of each index level and assessment criteria, we invited five experts from different fields, including pharmacological, biological, microbiological, zoological, and clinician experts. They were invited to answer the questionnaires anonymously to determine the weight of each index, and also provided assessment criteria for evaluation index (Table 1). Each expert's results were subjected to a consistency check, and all experts' results were combined and then subjected to a consistency check to ensure the objectivity and credibility of the data. The five experts selected for this study all have more than 10 years of professional work experience.

Table 1
Evaluation criteria for the skin wound infection model.

Indicators	Weight	Sub-indicators	Weight	Grade (R_i)				
				Poor	General	Medium	Good	Excellent
				(2 points)	(4 points)	(6 points)	(8 points)	(10 points)
Physical sign changes	0.0518	Weight changes	0.0204	weight gain $P > 0.05$	same weight	weight loss $P > 0.05$	weight loss $P < 0.05$	weight loss $P < 0.01$
		Feeding rate	0.0186	[80–100]%	[60–80]%	[40–60]%	[20–40]%	[0-20]%
		Sign of fatigue	0.0128	[0–1]	[1-2]	[2-3]	[4-5]	[3-4]
Appearance of skin lesions	0.2934	Redness and purulent secretion	0.0522	[0–1]	[1-2]	[2-3]	[3-4]	[4-5]
		Change of ulcer area	0.1505	increase very significantly $P < 0.01$	increase significantly $P < 0.05$	$P > 0.05$	reduce significantly $P < 0.05$	reduce very significantly $P < 0.01$
		Ulcer healing time	0.0907	[21-∞]	[1–7]	[14–21]	[7–14]	N/A
Morphological observation	0.3184	Skin histopathology	0.2483	[0–1]	[1-2]	[2-3]	[3-4]	[4-5]
		Vital organs	0.0701	N/A	5 groups $P < 0.05$	all groups $P > 0.05$	one group $P < 0.05$	two groups $P < 0.05$
Etiological examination	0.3364	Blood bacterial count	0.0891	decrease very significantly $P < 0.01$	decrease significantly $P < 0.05$	no significant change $P > 0.05$	increase significantly $P < 0.05$	increase very significantly $P < 0.01$
		Skin bacterial count	0.2473	decrease very significantly $P < 0.01$	decrease significantly $P < 0.05$	no significant change $P > 0.05$	increase significantly $P < 0.05$	increase very significantly $P < 0.01$

1,3,5,7,9: Intermediate values between adjacent scales values. Standard [0–5]:in which 0 mean health and 5 mean severe lesions.

(1) Pharmacological expert-40 years old-has 17 years professional knowledge
(2) Biological expert-38 years old-has 12 years professional knowledge
(3) Microbiological expert-50 years old-has 29 years professional knowledge
(4) Zoological expert-39 years old-has 15 years professional knowledge
(5) Clinician expert-43 years old-has 18 years professional knowledge

2.4. Construct pairwise comparison matrices

Once the hierarchy model was established, the decision-makers systematically evaluate its each factor by pairwise comparison. Pairwise comparisons were used to determine the relative importance of each indicator. For factor L and next hierarchical factor K, we use equation [28]

$$\text{matrix} = \alpha \begin{vmatrix} k_{11} & k_{12} & k_{13} & k_{14} & k_{15} \\ k_{21} & k_{22} & k_{23} & k_{24} & k_{25} \\ k_{31} & k_{32} & k_{33} & k_{34} & k_{35} \\ k_{41} & k_{42} & k_{43} & k_{44} & k_{45} \\ k_{51} & k_{52} & k_{53} & k_{54} & k_{55} \end{vmatrix} \quad (1)$$

to represent the relationships between M and N and establish the judgment matrix$_{\alpha}$. For quantitative judgment, this evaluation was performed by different experts in various fields, which was done with a 1–9 preference scale [29]. The definition and explanation of each priority level was presented in Table 2.

The pairwise comparisons have two principles:

The priority of each factor relative to itself is equal to 1.

If the priority of factor *i* factor *j* is equal to a_{ij}, then the priority of factor *j* to factor *i* should be equal to $\alpha_{ji} = 1/\alpha_{ij}$.

2.5. Carry out level sequence and check the consistency

After the pairwise comparison matrices being established, the characteristic root and characteristic vector of the matrix need to be calculated. Then, the weight of relative importance of any factor relative to the previous hierarchical factor can be calculated by Sum-product method, using formula [28] (2) $\overline{\alpha}_{ij} = \frac{\alpha ij}{\sum_{n} \alpha ij}$ and (3) $\bar{}\omega i = \sum \overline{\alpha} ij$. In which, α_{ij} is the various elements in matrix α, $\overline{\omega}_i$ is geometrical mean value of vector, ω_i is the weight of factor *i*. Then, the maximum characteristic value of matrix α is calculated by using formula [28] (4) $\lambda_{\max} = \frac{1}{n} \sum_{i=1}^{n} \frac{(A\omega)_i}{\omega_i}$. In which, $\lambda_{\max}$ is the maximum characteristic value of matrix α, other symbols have the same meanings as above.

Level simple sequence is used to determine weight (importance degree) between elements in a level and related element in higher level. The deviation from judgment matrix A's consistency is expressed by the following equation consistency index (CI) [28]. (5)$CI=(\lambda_{\max}\text{-}n)/(n\text{-}1)$, where n is the number of level.

Consistency ratio (CR) is used to estimate directly the consistency of pairwise comparisons, which is calculated by using formula [30](6)$CR=CI/RI$, If the CR is less than 0.1, the judgment matrix is consistent and the comparisons at same level are acceptable and logical. Random index (RI) is used to measure the judgment whether matrixes at different level are consistent or not. The RI (NA, NA, 0.58,0.9,1.12,1.24,1.32,1.41,1.45,1.49) values of 1–10 attributes.

2.6. Computer programming solution

Use Matlab software to input code for calculation:

Table 2
Pairwise comparison values.

Variables	Verbal terms	Explanation
1	Equally importance	Equal importance
3	Moderate importance	Moderate importance of one over the other
5	Strong importance	Essential or strong importance
7	Very strong importance	Very strong or demonstrated importance
9	Extreme importance	Extreme or absolute importance
2,4,6,8	Intermediate values between adjacent scales values	Intermediate values between the two adjacent judgments
1/2 to 1/9	Inversely preferred	Element *i* is the inverse of element *j*

A = [1 1/7 1/7 1/5; 7 1 1 3; 7 1 1 3; 5 1/3 1/3 1];
[v,d] = eig(A);
eigenvalue = diag(d); lamda = max (eigenvalue).
ci = (lamda-4)/3
cr = ci/0.90
w = v(:,1)/sum(v(:,1))

2.7. Scoring criteria for each indicator of skin wound infection model

Based on the cited standards with a five level ordinal scale, the comprehensive evaluation system of skin wound infection model was classified into 5 grades (Table 1).

2.8. Evaluation formula of animal infection model

After the animal skin infection model evaluation index system and its weight have been determined, different model schemes and grading criteria of the evaluation index can be used to score the objective index and the subjective index so as to evaluate the different model schemes and calculate by using formula [30] (7) $Q = \sum \omega_i \bullet R_i$. In which, Q was the mean total score, ω_i is the weight of factor i, R_i is the score of factor i.

2.9. The consistency check

The indicators used in this study were divided into five matrices and the relative weight of each indicators was determined by expert interview. The CR was equal to 0.04. The value of this ratio was smaller than 0.1 and it validated the consistency of the matrix of pairwise comparisons.

2.10. Weight of indicators

Once the weights of the index categories and the relative weights of the indicators in each category have been determined, the final weight of each indicator could be calculated (Table 1). This weight represented the importance of each in relation to the total set of indicators.

2.11. Animal experiment

The mice (18–22 g) were divided into nine groups. A full thickness of the excision wound of round or square in 1.5 × 1.5 cm area was created by using a blade, and scratch group was created by using needles within a defined 1.5 × 1.5 cm area. After that, except blank groups inoculated with 0.1 mL normal saline, others were inoculated with 0.1 mL saline containing 1.0×10^9 CFU or 1.0×10^8 CFU of *S. aureus* [31,32] (Strain ATCC6358 [29,33], preserved in the department of pathogenic microbiology, Guangdong Pharmaceutical University. When cultures reached an optical density (OD_{600}) of 0.75, the corresponded bacterial cell density is 10^8 CFU/mL).

The rats (180–220 g) were divided into six groups. A full thickness of the excision wound of round area in 2.0 cm or 1.5 cm diameter was created by using a blade, except blank groups inoculated with 0.2 mL normal saline, others were inoculated with 0.2 mL saline containing 2.0×10^9 CFU or 2.0×10^8 CFU of *S. aureus*.

The animals were observed for signs of fatigues, feeding rate, and weight changes. The infection sites were inspected by blind test, and the grades of swelling and erythema were scored by using a clinical standard: 0, 1,2,3,4, 5, in which 0 mean healthy skin and 5 mean massive swelling or redness. The size of the wound area was measured with calliper, and the wound area was calculated:$S = \pi\bullet(L/2)\bullet(W/2)$, in which L was the length and W was the width. Then the healing rate of the ulcers were calculated by formula $p=(1\text{-}S_i/S_0) \times 100\%$, where S_i was the ulcer area on day i, and S_0 represented the area of primitive ulcer. We also recorded the duration of wound infection. At last the animals were euthanized and tissue sampling was carried out from the wound sites on days 2 or 3, 8 and 11 for histology inspection and bacteria reverse culture. Blood was collected from the front of the eyelid on days 2 or 3. The issue homogenate (0.1 g tissue was placed in 5 mL of sterile saline and vortexed for 1 min to disperse the bacteria) and the blood were used as a stock solution respectively, which were sequentially diluted to 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 1 mL dilutions were respectively taken in a disposable plate with mannitol salt agar (HKM, Guangzhou, China), two plates were set for each dilution, incubating in 37 °C for 48 h, the plate counting method was adopted, the plate with the number of colonies in the range of 30–300 were selected to count.

Animal welfare and experimental procedures were strictly observed, in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments, or the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No.8023, revised 1978) on Nov. 10, 2017. The animals (half male and half female) were obtained from the Medical Experimental Animal Center of Guangdong Province (China). The experimental scheme of animal study was approved by the ethics committee of Guangdong Pharmaceutical University (No. gdpulacSPF2017036).

2.12. Statistical analysis

Data were expressed as mean ± SD, with statistics carried out in the SPSS 19.0. Shapiro Wilk test was used to check whether the

data is normally distributed. One-way analysis of variance (ANOVA) Dunnett's multiple comparison was used to compare multiple infection groups with blank group. The values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. Skin ulcer infection model in mice

3.1.1. Physical sign changes

24 h after infection, the feeding rate of mice in each model group was affected, as time went on, the feeding rate gradually returned to normal (Fig. 2a). The mental states of mice were similar. 24 h after infection, compared with respective blank groups, the weight of mice in round high and low concentration groups decreased obviously ($P < 0.05$ or $P < 0.01$), the weight of mice in square high and low concentration groups also decreased, but with no statistical significance, and there was no significant difference between scratch high or low concentration group and scratch blank group (Fig. 2b).

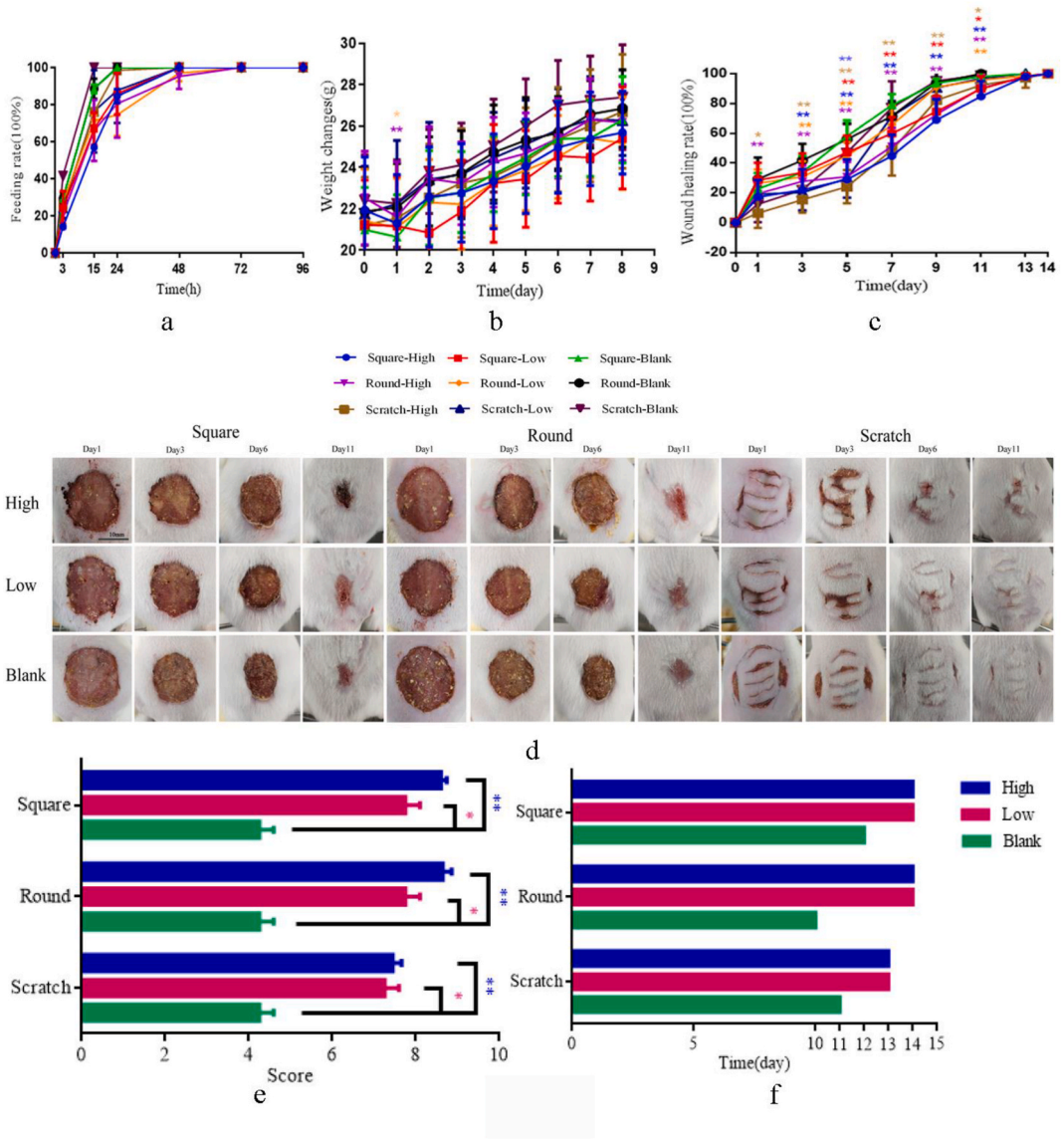

Fig. 2. Physical sign changes and appearance of skin lesions in mice. (a)Changes of feeding rate in mice, mainly observed four days after infection. (b)Weight changes in mice, mainly observed one week after infection. (c)Changes of ulcer area healing rate in mice, the measurement was carried out throughout the experimental period. (d)Appearance of skin ulcer in mice, the photos of 1st, 3rd, 6th and 11th day were selected to show the gradual healing process of ulcer. (e) Score of redness and purulent secretion in each group. (f) The ulcer healing time in each group. Comparing with the blank group, $*P < 0.05$,$**P < 0.01$, ($\bar{x} \pm s$, $n = 22$).

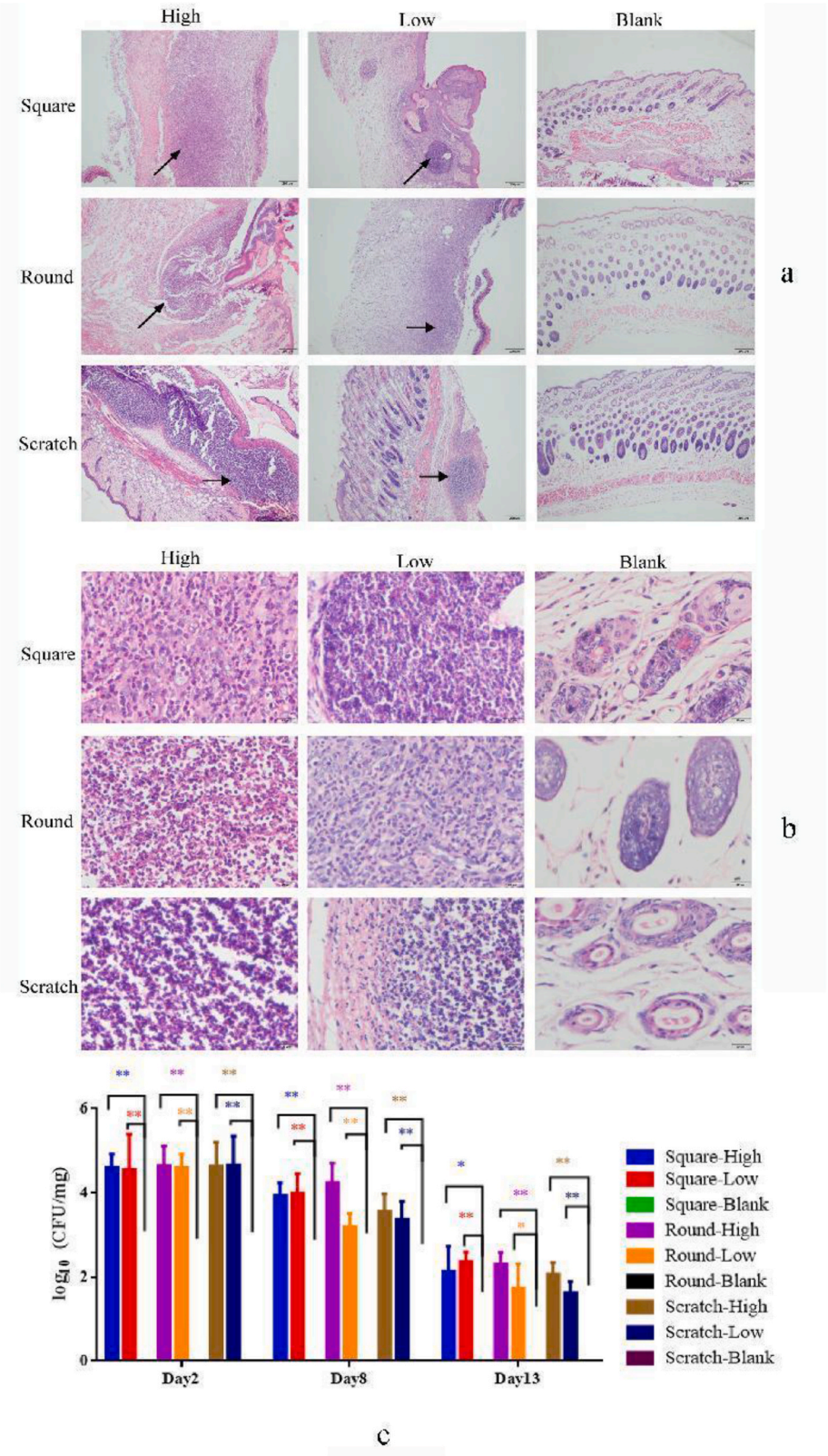

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Fig. 3. Morphological observation and etiological examination in mice. (a)Pathological section of animal skin (HE staining, × 40). The black arrowhead indicated infiltration of inflammatory cells. (b)Pathological section of animal skin (HE staining, × 400). (c)The bacteria content of skin in mice. Comparing with the blank group, $^{*}P < 0.05$,$^{**}P < 0.01$, ($\bar{x} \pm s$, n = 6–8).

3.1.2. Appearance of skin lesions

48 h after infection, we observed the appearance of skin lesion in each group. In square or round damage groups, the mice all showed marked redness and purulent secretions at the site of infection, especially the high concentration groups were more serious (Fig. 2d). The high concentration of scratch group also showed obvious redness and purulent secretions, but the infection site could not be sized very well. The healing rate of ulcer was an indicator that directly reflected the lesion appearance. The appearance of skin lesions indicators results were showed in Fig. 2c, e, 2f.

3.1.3. Morphological observation

After injury and infection, the structure of the skin layers in high concentration of the square group completely disappeared, and a large number of inflammatory cells infiltrated around the ulcers in a massive distribution. The hair follicles and sebaceous glands in low concentration of the square group were present, and a small number of inflammatory cells around the ulcer infiltrated into a scattered distribution. In the low concentration of round group, the structure of each layer of the skin completely disappeared, and a large number of inflammatory cells infiltrated into strips around the ulcer. The structure of the layers in high concentration of round group completely disappeared; the inflammatory cells in the periphery of the ulcer infiltrated into a polydisperse distribution. In the low and high concentration of scratch groups, the structure of each layer of the skin is clear, but inflammatory cells infiltrate around the ulcer into massive distribution (Fig. 3a and b).

We also checked the animal's organ index such as heart, liver, spleen, lung and kidney. But the results showed that only the spleen organ index decreased in Square high-concentration group, and there was no significant difference between the other model groups and the blank group, which indicated that these organs may not change significantly during the process of skin ulcer lesion.

3.1.4. Etiological examination

48 h after infection, we observed bacterial contents of animal blood in each group. The results showed that there was no bacteria in the blood. However, the bacterial contents in mice skin of model groups increased significantly, especially on the second day after infection (Fig. 3c). These results indicated local skin was the main site of infection in the model of infective skin ulcer.

3.1.5. The total score

The above evaluation indexes were scored according to the evaluation criteria for the skin wound infection, and the comprehensive score of each index was calculated. The final result showed that the Round-High group got the highest score (Table 3), which suggested that this model established by a round ulcer with high concentration of 1.0×10^{10} CFU/mL *Staphylococcus aureus* (0.1 mL) may be the best model of infection skin ulcer in mice.

3.2. Skin ulcer infection model in rats

3.2.1. Physical sign changes

Similar to the mice test, 24 h after infection, the feeding rate of rats in each model group was affected, but over time, the feeding rate gradually returned to normal (Fig. 4a). Compared with respective blank group, the weight changes of rats in 2.0 cm or 1.5 cm high and low concentration groups slowed down, only 2.0 cm-Low group with statistical significance ($P < 0.05$) (Fig. 4b).

3.2.2. Appearance of skin lesions

On the first day after modeling, most of the back wounds had purulent secretions, peripheral tissue edema, redness and swollen wounds, inflammation of the edge of the wound, and some animals had blood clots on the back. Among them, in the 2.0 cm-High group, there was a large amount of purulent secretions on the wound surface, the surrounding area of the wound was blackened, and the wound depression was relatively full. A large amount of purulent secretions also appeared in the wound of the 1.5 cm-High group, which was lighter in color than the wound of 2.0 cm group. In the blank group, there was no tumor at the wound surface margin, with a small amount of purulent secretions, and the wound was fleshy pink. In the 4th days, the wound surface was moist, and the infection of the wound was more serious. On the 5th day, the wounds of each model group gradually began to scabs, and the wounds were significantly reduced after 7 days. On the 8th day, some animals began to shed scabs, the wound area was significantly reduced, and the new skin surface was flesh pink. The wound healed more than 80% after 12 days. However, on the whole, the animals in the blank groups healed faster, and the rate and state of healing were significantly better than those in the model groups (Fig. 4d). There was a significant difference in the healing rate between the blank groups and the model groups. The healing rate of ulcer area in 1.5 cm-High group was significantly different from that in the blank group on day 3–13 ($P < 0.05$ or $P < 0.01$), and the 1.5 cm-Low group was significantly different from the blank group on day 5–7 and day 13–15 ($P < 0.05$). The healing rate of ulcer area in the 2.0 cm-High group was significantly different from that in the blank group on day 5–11 ($P < 0.05$ or $P < 0.01$). There was no significant difference in the degree of recovery after day 15. The healing rate changes and the healing time of each group were showed in Fig. 4c, e, f.

Table 3
Comprehensive score for each group.

Indicators	Weight of indicator categories	Sub-indicators	Final weight of all indicators	Comprehensive score for each group in mice						Comprehensive score for each group in rats			
				Square-High	Square-Low	Round-High	Round-Low	Scratch-High	Scratch-Low	2.0 cm-High	2.0 cm-Low	1.5 cm-High	1.5 cm-Low
Physical sign changes	0.0518	Weight changes	0.0204	6	5	10	8	2	2	5	9	5	6
		Feeding rate	0.0186	2	2	2	3	2	1	1	1	1	1
		Sign of fatigue	0.0128	8	6	8	8	8	8	6	6	6	6
Appearance of skin lesions	0.2934	Redness and purulent secretion of skin	0.0522	9	8	9	8	7	7	8	7	8	6
		Changes of ulcer area	0.1505	10	8	10	10	8	6	3	3	3	3
		The heal time of ulcer	0.0907	7	7	7	7	7	7	5	6	7	7
Morphological observation	0.3184	Skin histopathology	0.2483	4	4	5	5	5	4	4	4	5	3
		Observation of vital organs	0.0701	8	6	6	6	6	6	6	6	6	6
Etiological examination	0.3364	Blood bacterial count	0.0891	5	5	5	5	5	5	5	5	5	5
		Skin bacterial count	0.2473	7	7	7	7	7	7	7	7	7	7
Total score	1		1	6.6023	6.0629	6.792	6.7176	6.2234	5.6555	5.1104	5.2305	5.5401	4.9595

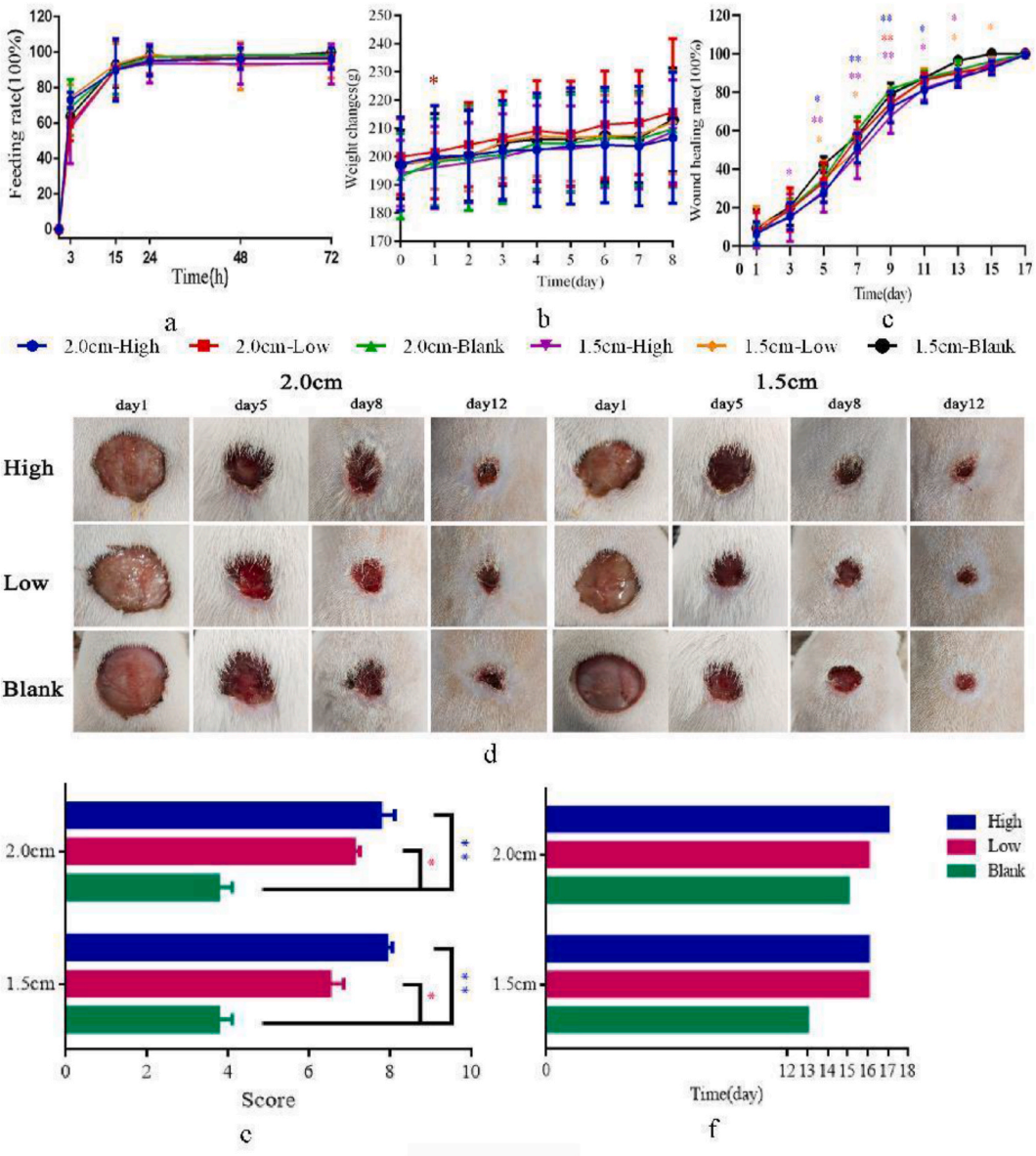

Fig. 4. Physical sign changes and appearance of skin lesions in rats. (a) Changes of feeding rate in rats, mainly observed three days after infection. (b) Weight changes in rats, mainly observed one week after infection. (c)Changes of ulcer area healing rate in rats, the measurement was carried out throughout the experimental period. (d)Appearance of skin lesion in rats, the photos of 1st, 5th, 8th and 12th day were selected to show the gradual healing process of ulcer. (e)Score of redness and purulent secretion in each group. (f) The ulcer healing time in each group. Comparing with the blank group, $^{*}P < 0.05$,$^{**}P < 0.01$, ($\bar{x} \pm s$, $n = 18$).

3.2.3. Morphological observation

The structure of rats skin in 2.0 cm-High group, 2.0 cm-Low group, 1.5 cm-High group completely disappeared, and a large number of inflammatory cells infiltrated into the subcutaneous tissue and distributed in a massive or scattered manner, and the infiltration of inflammatory cells in 1.5 cm-Low group was slightly lighter. However, in the two blank groups, the structure of rats skin did not completely disappear, and hair follicles and sebaceous glands were still visible, and subcutaneous tissue was infiltrated by a small number of inflammatory cells (Fig. 5a,b).

And the main organs of rats such as heart, liver, spleen, lung and kidney index were removed and weighed, then calculated the organ index. But there was no significant difference between each model group and the blank group, this result was close to that of mouse experiment.

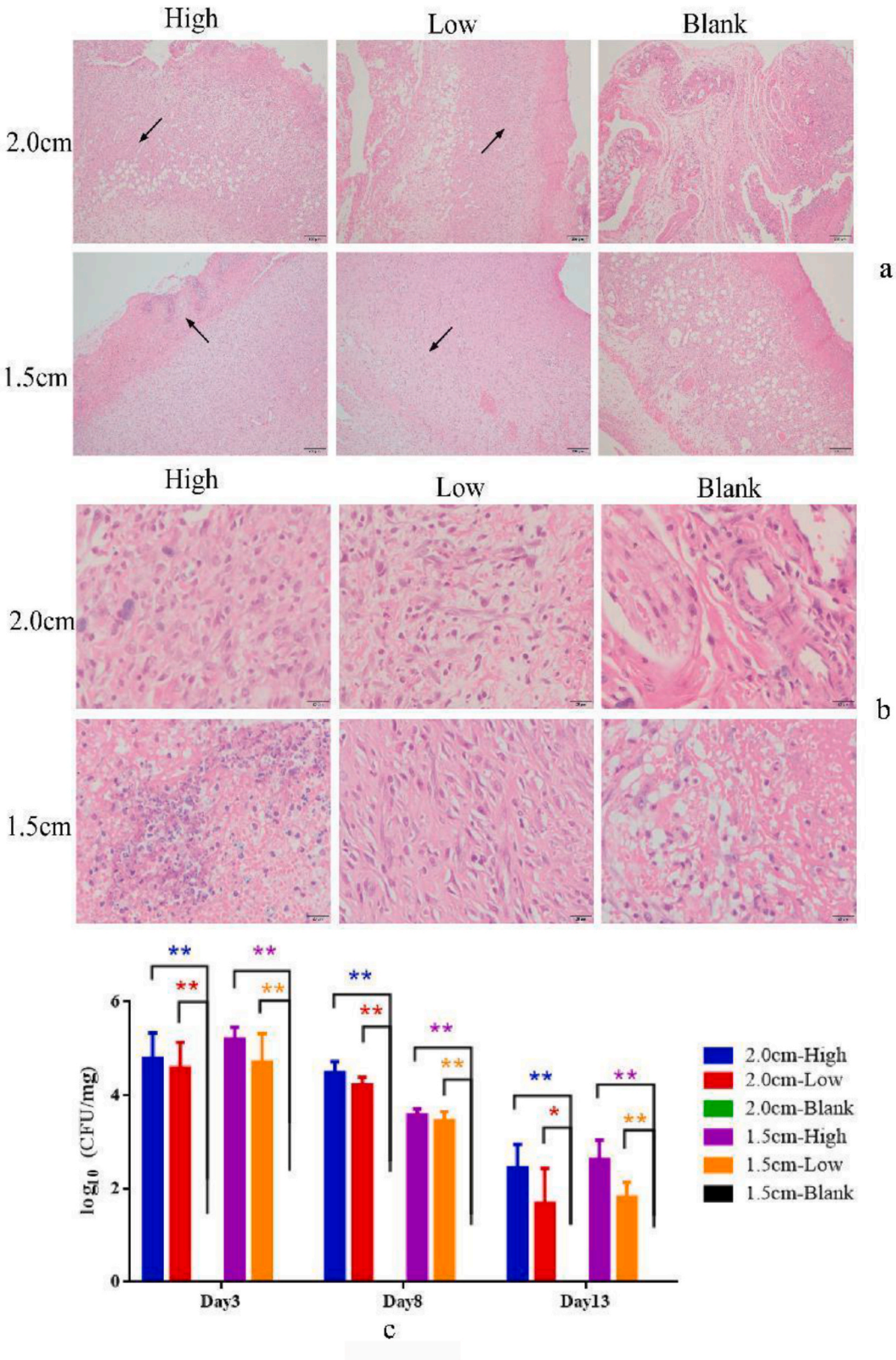

Fig. 5. Morphological observation and etiological examination in rats. (a)Skin pathological sections of rats (HE staining, × 40). The black arrowhead indicated infiltration of inflammatory cells. (b)Skin pathological sections of rats (HE staining, × 400). (c)The bacteria content of skin in rats. Comparing with the blank group, $^{*}P < 0.05$,$^{**}P < 0.01$, ($\bar{x} \pm s$, n = 6–8).

3.2.4. Etiological examination

There was no bacteria in the blood, and there was no significant difference in the blood bacterial content between the model group and the blank group. And the bacterial content in the local skin of the four model groups first increased rapidly after infection, and then gradually decreased with the passage of time, but there was still significant difference between the model groups and the blank groups at the end of the experiment (Fig. 5c).

3.2.5. The total score. The evaluation indexes were scored according to the assessment criteria, and the comprehensive score of each index was calculated and showed in Table 3. In rat models, the 1.5 cm-High group got the highest score, which suggested that 1.5 cm round ulcer with high concentration of 1.0×10^{10} CFU/mL *S. aureus* (0.2 mL) may be the best model of infection skin ulce.

4. Discussion and conclusion

A useful and stable animal model can provide reliable and reproducible results. This study showed that one of the most important steps in the evaluation of skin disease models was to establish the weights of model quality parameters. AHP is a multi objective decision-making (evaluation) analysis technique that combines qualitative and quantitative analysis, AHP combined with Delphi method has the advantage of solving problems qualitatively and quantitatively by combining the experience and judgment of experts into the model with quantitative index [34–37]. Therefore, we introduced this method to establish the weights of parameters for skin infection model.

At the index layer, the etiology parameters had the greatest priority, because of the dominating index in skin infections [38,39], they were followed by morphology parameters, appearance parameters, while the physical parameters had the least priority. Therefore, for the skin microbial infection models, the etiology and morphological parameters should be evaluated in the most preferred way. In relation to the physical parameters, the evaluators gave weight changes the greatest priority, it suggested that the change of animal weight was an important parameter reflecting the occurrence and maintenance of skin abscess infection model, which may be due to the loss of food and other uncomfortable signs (such as fatigue) caused by skin lesions. With respect to appearance parameters, the evaluators ranked the parameter of change of ulcer area as the highest, which implied that change of ulcer area was also a significant parameter to evaluate whether the ulcer model is effective and successful. In morphology parameters, the evaluators gave skin histopathology the greatest priority. And in relation to etiology parameters, skin bacterial count received the greatest weight, which indicated that the evaluation of animal models of skin microbial infection may be mainly based on local skin infection. Based on the above evaluation parameters and comprehensive system, we firstly evaluated six kinds of ulcer models in mice, and confirmed that mice damaged with a round wound and inoculated with 1.0×10^{10} CFU/mL concentration of *S. aureus* (0.1 mL) was the best model. After the round damage mode was determined, we continued to investigate the injury area (2.0 cm or 1.5 cm) and inoculation concentration in rats. Then according to the evaluation system, among the four infection ulcer models in rats, the model whose lesion caused by a round wound of 1.5 cm in diameter with 1.0×10^{10} CFU/mL (0.2 mL) was determined to be the best.

In summary, we have established a new model-evaluation system for skin wound infection models by AHP and Delphi method, and provided two examples for the evaluation of skin diseases animal models. In contrast to previously described models of skin infection [9,40,41], the evaluation system in our model is quantified and more systematic. In order to promote the application, the evaluation methods and standards still need to be further improved.

Declarations

The experimental scheme of animal study was approved by the ethics committee of Guangdong Pharmaceutical University (No. gdpulacSPF2017036).

Declaration of competing interest

The authors declare no conflict of interest.

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