Appendix 3

Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine

Human Subject Research Protocol

(For Prospective Studies)

Study Title:	Efficacy and Safety Evaluation of Palmitoleic Acid in the Prevention of Pressure Injuries
Protocol Number:	
Principal Investigator(s):	Zheng Jie, Yuan Yongyong
Department:	Department of Dermatology, Ruijin Hospital affiliated to Shanghai Jiao Tong University School of Medicine
起止年限:	January 2026 to December 2026

Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine

December 8, 2025

Version: 3.0

1. Study Synopsis

1.1 Synopsis

Study Title:	Efficacy and Safety Evaluation of Palmitoleic Acid Oil Composition in the Prevention of Pressure Injuries
Brief Description：	This is a prospective, single-center, self-controlled trial designed to evaluate the efficacy and safety of topical Palmitoleic Acid (POA) application in preventing Pressure Injuries (PI). The study targets hospitalized patients at high risk for PI or with Stage I PI. In addition to standard care, the intervention involves applying POA to the sacral region four times daily for two weeks. Efficacy and safety will be comprehensively assessed by monitoring PI occurrence and reversal, skin physiological parameters (SEM, $TcPO_2$), and adverse events.
Study Objectives:	To evaluate the efficacy and safety of Palmitoleic Acid Oil Composition in preventing Pressure Injuries, providing empirical data to support its clinical application.
Study Population：	Hospitalized patients at high risk for Pressure Injury or with Stage I Pressure Injury.
Study Site/Location:	Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine
Study Intervention:	In addition to routine standard care, the intervention involves applying POA to the patient's sacral region (1 mL per application, four times daily, for 14 days). Primary outcome measures include the PI prevention rate during the intervention period, the reversal rate of Stage I PI, and dynamic changes in SEM values and transcutaneous oxygen pressure ($TcPO_2$).
Study Duration:	14 days
Subject Participation Time:	The skin condition of the subject's sacral region will be photographed, and SEM values and transcutaneous oxygen pressure at the pressure site will be measured, along with dermoscopy, at baseline and on Day 7 and Day 14 after the start of the experiment.

2. Study Background

2.1 Study Significance

To evaluate the role of Palmitoleic Acid oil composition in preventing Pressure Injuries, providing effective prevention strategies and evidence for clinical practice.

2.2 Background

Pressure Injury (PI), also known as pressure ulcer or decubitus ulcer, is a pathological process where local tissue ischemia and hypoxia, caused by pressure or a combination of pressure and shear forces on the skin and underlying soft tissue, leads to tissue damage. Clinical manifestations range from localized tissue damage with intact skin to open ulcers, often accompanied by severe pain. In recent years, the incidence of PI has been increasing, with a global prevalence of 12.8% among hospitalized adult patients. PI is a major cause of prolonged hospital stays, reduced quality of life, and increased mortality and healthcare costs.

Current PI prevention strategies primarily include clinical risk assessment, repositioning, pressure-relieving devices, and nutritional support. However, these measures often only delay ulcer progression without fundamentally addressing the problem. Existing therapeutic or preventive strategies for PI show unsatisfactory clinical efficacy, and PI incidence remains high, partly due to limited understanding of its underlying mechanisms. To date, ischemia-reperfusion (I/R) injury is considered a primary determinant in PI formation. I/R injury refers to cellular damage caused by the restoration of blood flow to previously ischemic and hypoxic tissues. This process may cause extensive cellular damage by activating leukocytes and inducing oxidative stress. Therefore, interventions targeting this mechanism, particularly mitigating I/R injury through antioxidant and anti-inflammatory pathways, are considered potential strategies to overcome current PI prevention challenges.

Palmitoleic Acid (POA) is an ω-7 monounsaturated fatty acid that has recently gained attention for its significant biological activities, including anti-inflammatory, antioxidant, and tissue repair-promoting effects. Basic research suggests that POA may have a potential protective effect against I/R injury by modulating inflammatory factor expression and reducing oxidative stress. However, these valuable findings have not been fully translated and validated in the clinical practice of PI prevention. High-quality clinical evidence regarding the efficacy

and safety of topical POA application for PI prevention remains lacking.

In summary, facing the severe clinical challenges and high socioeconomic burden posed by PI, there is an urgent need to explore and validate novel, targeted preventive strategies based on a deeper understanding of its core mechanism—I/R injury. This study aims to evaluate the clinical efficacy and safety of topical Palmitoleic Acid application in preventing PI. It not only holds the potential to provide an innovative intervention for PI prevention but will also offer valuable clinical evidence for understanding the role of fatty acids in tissue protection.

2.3 Expected Outcomes

To evaluate the effectiveness and safety of the skin care intervention in preventing pressure injuries, providing scientific evidence and clear guidance for clinical nursing practice.

2.4 Risk/Benefit Assessment

2.4.1 Known Potential Risks

During the study, some, all, or none of the following adverse events, risks, discomforts, or inconveniences may occur:

1. This study involves a Palmitoleic Acid care product, preliminarily assessed as safe with no foreseeable risks. However, due to individual differences, allergic reactions such as redness or wheals may still occur.
2. Some questions asked during the study may make the subject's guardian uncomfortable. The guardian can refuse to answer such questions and may also withdraw from the study.

2.4.2 Known Potential Benefits

For the subject, it may help maintain appropriate skin moisture and prevent the occurrence of pressure injuries.

2.4.3 Potential Risk/Benefit Assessment

Palmitoleic Acid is a safe component with rare allergic reactions, yet it can effectively prevent pressure injuries, reducing the negative impacts such as patient suffering, infection, and increased medical costs associated with PI occurrence.

3. Principal Investigator Information

3.1 Principal Investigator Name, Qualifications, Contact Information

Zheng Jie, Chief Physician, 18917762309; Yuan Yongyong, Associate Chief Nurse, 18917762651

3.2 Key Personnel Information

No.	Name	Gender	Age	Title	Specialty	GCP Trained ?	Role in Study（eg. PI、sub-I、CRC）
1	郑捷	男	70	Chief Physician	Dermatology & Venereology	Yes	PI
2	袁勇勇	女	42	Associate Chief Nurse	Nursing	Yes	PI
3	张婷	女	40	Nurse-in-Charge	Nursing	Yes	sub-I
4	乐云辰	男	42		Metabolomics	Yes	CRC
5	汤姜杨	女	28		Pharmacy	Yes	CRC

4. Study Objectives

To clarify the efficacy and safety of Palmitoleic Acid Oil Composition in preventing pressure injuries and provide empirical data support.

5. Study Design

5.1 Overall Design

- **Hypothesis**：Palmitoleic Acid oil composition is effective in preventing pressure injuries and superior to standard care.
- **Study Phase:** Prospective, single-center, self-controlled trial (pre-post intervention).
- **Study Design:**The study targets hospitalized patients at high risk for PI or with Stage I PI. In addition to routine care, POA is applied to the sacral region four times daily for two weeks. Efficacy and safety are comprehensively evaluated by monitoring PI occurrence, reversal, skin

physiological parameters (SEM, $TcPO_2$), and adverse events.

- **Methods to Reduce Bias:** Standardized training and management for assessment and operations.
- **Study Groups & Intervention Duration:** Each subject's intervention period is 2 weeks, with follow-up until Day 28.
- **Multicenter?:** No, this is a single-center study.
- **Study Intervention Method**：In addition to routine care, apply POA to the sacral region four times daily for two weeks. Apply 1 mL of the care oil four times daily (8:00, 12:00, 16:00, 20:00), gently massaging until fully absorbed.

5.2 Definition of Study Endpoint

The study endpoint is reached when the subject completes all stages of the study or follow-up according to the protocol, or withdraws informed consent.

5.3 Sample Size Calculation

This is a prospective study with no prior reference data, so the sample size is not calculated based on statistical hypotheses. A total of 60 hospitalized patients at high risk for PI or with Stage I PI are planned to be enrolled to preliminarily assess the effect and safety of the intervention.

6. Study Subjects

6.1 Inclusion Criteria

①Hospitalized patients at high risk for Pressure Injury or with Stage I Pressure Injury.

②The subject and/or their guardian voluntarily agree to use the test product as directed by the physician throughout the study and sign the informed consent form.

6.2 Exclusion Criteria

①Patients currently participating in other clinical studies or who have participated in other clinical studies within the past 3 months.

②Patients who have used other moisturizing products or medications within 3 days prior to enrollment.

③Subjects with poor compliance, known inability to attend visits on time, or unwillingness to comply with the study schedule during the study period.

④Patients with existing Stage II or higher Pressure Injury, or immobilized patients.

6.3 Subject Recruitment

Plan to recruit 60 hospitalized patients at high risk for PI or with Stage I PI at Shanghai Jiao Tong University School of Medicine Affiliated Ruijin Hospital to participate in this prospective study.

6.4 Method of Subject Allocation

This study adopts a self-controlled (pre-post intervention) design. All enrolled subjects receive the unified intervention and are compared before and after the intervention.

7. Study Intervention

7.1 Administration of Study Intervention

7.1.1 Description of Study Intervention

Application of Palmitoleic Acid oil composition to the pressure site (sacral region) in addition to implementing standard Pressure Injury prevention care measures.

7.1.2 Dosage and Administration Method

Each application uses 1 mL, gently massaged until fully absorbed. Applications are performed four times daily according to the schedule (8:00, 12:00, 16:00, and 20:00).

7.2 Preparation/Handling/Storage/Responsibility

7.2.1 Responsibility

After enrollment into the intervention group, the investigator provides the Palmitoleic Acid oil and applies 1 mL to the patient's sacral region four times daily. The Palmitoleic Acid oil is stored by the investigator.

7.2.2 Composition, Appearance, Packaging, and Labeling

- Product Name: Rui Ke Hu Oxygen-Enhancing Care Oil
- Filing Number: Hu G Zhuang Wang Bei Zi 2025000921
- Filing Entity: Shanghai Ruike Yi Cosmetics Technology Co., Ltd.
- Filing Entity Address: Room 415, 4th Floor, Building 3, No. 105 Sinan Road, Huangpu District, Shanghai

- Manufacturer Name: Yatsen Biotech (Guangzhou) Co., Ltd.
- Manufacturing Address: Building A, 1st-2nd Floor of Building B, 3rd Floor of Building C, No. 219 North Mingzhu Avenue, Chengjiao Street, Conghua District, Guangzhou
- Full Ingredient List: Zea Mays (Corn) Oil, Hippophae Rhamnoides Fruit Oil, Pentaerythrityl Tetrakis(bis(2,2,6,6-tetramethyl-4-piperidyl) sebacate)
- Color/Appearance: Yellow transparent oily liquid

7.2.3 Product Storage and Stability

Store in a dry, ventilated place at room temperature, protected from light. Avoid high temperatures and direct sunlight.

7.3 Measures to Reduce Bias:

This trial uses a self-controlled study design. Standardized management of assessments and operations is intended to be employed to reduce bias.

7.4 Follow-up and Compliance

- Follow-up: The skin condition of the subject's sacral region will be photographed, and SEM values and transcutaneous oxygen pressure at the pressure site will be measured, along with dermoscopy, at baseline and on Day 7 and Day 14 after the start of the experiment.
- Compliance: For hospitalized patients, the investigator applies 1 mL to the patient's sacral region four times daily to prevent the patient and/or family from forgetting to apply the product.

7.5 Study Intervention Commitment

Follow the study protocol to monitor the patient's pressure site SEM value, transcutaneous oxygen pressure, transcutaneous carbon dioxide pressure, and skin color at baseline, Day 7, and Day 14. Record all data in the "Pressure Injury Prevention Study Patient File" for statistical analysis. The "Pressure Injury Prevention Study Patient File" will be retained for 5 years after the study concludes.

7.6 Study Schedule

Time Point / Item	Screening Period	Treatment Period		Follow-up Period
	Baseline	Day 7	Day 14	Day 28
Informed Consent	×			
Demographic Data	×			
Medical History Collection	×			
Inclusion/Exclusion Criteria	×			
SEM Value	×	×	×	
Efficacy Observations				
Transcutaneous O_2 Pressure	×	×	×	
Skin Color	×	×	×	
Pressure Injury Occurrence	×	×	×	×
Other Observations				
Adverse Event Observation		×	×	

8. Study Intervention Discontinuation and Subject Withdrawal/Discontinuation

8.1 Study Intervention Discontinuation

The study intervention will be discontinued if the patient's condition changes and they cannot cooperate with the collection of various efficacy indicators. Pressure injury occurrence will be recorded after study discontinuation.

8.2 Subject Discontinuation/Withdrawal

- The investigator may discontinue or withdraw a subject under the following circumstances:
- Pregnancy
- Significant non-compliance with the study intervention
- If clinical side effects or other clinical conditions arise such that continued participation is no longer in the subject's best interest
- Disease progression requiring discontinuation of the study intervention
- The subject meets an exclusion criterion (newly occurring or confirmed)
- The subject is unable to receive the study intervention for a certain period
- The reason for subject discontinuation/withdrawal should be recorded on the Case Report Form (CRF). Subjects who sign the informed consent but do not receive the study intervention will be replaced. Subjects who sign the informed consent, receive the study intervention, and subsequently withdraw may or may not be replaced.

9. Study Endpoint Evaluation

9.1 Primary Endpoint Evaluation

①Pressure Injury prevention rate

②Stage I Pressure Injury reversal rate

9.2 Secondary Endpoint Evaluation

①Transcutaneous oxygen pressure ($TcPO_2$)

②Sub Epidermal Moisture (SEM)value

③ Skin irritation reaction

9.3 Safety Evaluation

Observe and record any adverse events (AEs), and evaluate the outcome of the AEs.

9.3.1 Adverse Event (AE) Definition

An Adverse Event is any untoward medical occurrence in a clinical trial subject administered the test product, which does not necessarily have a causal relationship with the product's use. Regardless of its relationship to the product, an AE can be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease. Pre-existing conditions that worsen during the trial should also be reported as AEs. However, natural worsening of the symptoms of the target indication is not considered an AE.

9.3.2 Serious Adverse Event (SAE) Definition

A Serious Adverse Event is any untoward medical occurrence that during the trial requires hospitalization, prolongs hospitalization, results in persistent or significant disability/incapacity, is life-threatening, results in death, or causes a congenital anomaly/birth defect.

9.3.3 AE Classification

9.3.3.1 Event Severity

Mild: Tolerable by the subject, does not affect treatment, does not require special treatment, and has no impact on the subject's recovery.

Moderate: Difficult for the subject to tolerate, requires special treatment, and has a direct impact on the subject's recovery.

Severe: Life-threatening to the subject, causing death or disability, requiring immediate emergency treatment.

9.3.3.2 Relationship to Study Intervention

Related: Caused by the use of the care oil.

Unrelated: Not caused by the use of the care oil.

9.3.4 Timing, Frequency, Follow-up, and Outcome of AE Assessment

Researchers will be responsible for monitoring subject AEs every 3 days, i.e., observing and recording during each efficacy measurement, and comparing with the last assessment and baseline indicators.

9.3.5 Adverse Event Reporting

After each visit, the researcher will assess, grade, and record AEs. If a Serious Adverse Event (SAE) occurs during a visit, the data will be transmitted to the Shanghai Ruijin Hospital Ethics Committee.

9.3.6 Serious Adverse Event Reporting

If an SAE occurs during the trial, regardless of its relationship to the test product, the used product should be sealed immediately, first-aid measures taken, and reported within 24 hours to the sponsor's responsible person, monitor, and Ethics Committee. If related to the product, it should also be reported to the National Medical Products Administration (NMPA) Drug Registration Department. An SAE report form will be completed.

10. Statistical Analysis

Normality of continuous variables (including SEM, transcutaneous oxygen pressure [$TcPO_2$], Braden Scale score, and SpO_2) will be assessed using the Shapiro-Wilk test. For data conforming to a normal distribution, overall comparison will be performed using repeated-measures one-way Analysis of Variance (ANOVA). If the sphericity assumption is violated, the Geisser-Greenhouse correction will be applied. Pairwise comparisons between groups will be conducted using Tukey's post-hoc test. For data not conforming to a normal distribution, the Friedman test will be used for analysis, with pairwise comparisons conducted using Dunn's post-hoc test.

Categorical variables will be expressed as frequency and percentage (n [%]). For primary clinical outcomes (Pressure Injury incidence rate and Stage I reversal rate), 95% confidence intervals will be calculated using the Clopper-Pearson exact method. The correlation between Braden Scale scores and SEM values will be analyzed using Spearman's rank correlation.

All statistical tests will be two-sided, with a $P < 0.05$ considered statistically significant. Data analysis and graph plotting will be performed using GraphPad Prism software (version 9.0).

11. Supporting Documents and Notes

11.1 Informed Consent Process

Informed consent must be obtained before a subject agrees to participate in the study and is an ongoing process throughout the study. The informed consent form, approved by the Ethics Committee, should be read by the subject. The investigator will explain the study procedures and

answer the subject's questions, informing them of potential risks and their rights. The subject may discuss participation with family or guardians before agreeing. The investigator must inform the subject that participation is voluntary and that they may withdraw at any time during the study. A copy of the informed consent form may be provided to the subject. The subject's rights and welfare will be protected, and it will be emphasized that the quality of their medical care will not be affected by refusal to participate.

11.2Privacy Protection

All information disclosed or provided by the sponsor (or any company/institution representing the sponsor) or generated during the clinical trial, including but not limited to the trial protocol, CRFs, Investigator's Brochure, and results obtained during the trial, shall be kept confidential. The investigator and their subordinates agree to maintain confidentiality and shall not disclose information to any third party without the sponsor's prior written approval. However, specific permission is granted to submit this clinical trial protocol and other necessary documents to the Ethics Committee, whose members have the same confidentiality obligations.

Assisting investigators shall assume the same confidentiality obligations as the investigator. The investigator shall inform assisting investigators of the confidential nature of the clinical trial. The use of this information by the investigator and assisting investigators shall be limited to the purposes of the clinical trial and is prohibited for personal or third-party purposes.

11.3 Specimen and Data Collection and Use

All subject data are strictly confidential. As all medical information will become part of the physician's medical records, only authorized personnel may access the subject's medical records to verify the accuracy of collected information and ensure the study proceeds properly. The results of this project may be presented at scientific or medical conferences or published in scientific or medical journals. In all such processes, no personally identifiable information of the subject will be used unless required by law. All recorded photographs involving the head and face will undergo image masking.

11.4 Quality Control and Quality Assurance

11.4.1 **Quality Control**

11.4.1.1 **Qualifications of Study Site and Investigators:** Investigators participating in this

study must undergo qualification review and possess the professional background and capability to conduct clinical trials.

11.4.1.2 **Investigator Training:** Before the clinical trial begins, training will ensure that research personnel fully understand and recognize the clinical trial protocol and specific content of each indicator; ensure subject and investigator compliance with the trial protocol; and ensure tracking, follow-up, recording, and reporting of adverse events.

11.4.2 **Quality Assurance**

The human subject research protocol must be submitted to the Ethics Committee for approval before execution; clinical trial statistical analysis plan.

11.4.3 **Informed Consent and Informed Consent Form**

The investigator is responsible for explaining the purpose, methods, benefits, and potential risks of this clinical trial to each subject and obtaining the signed informed consent form from the subject participating in the clinical trial. Informed consent must be obtained before any procedure related to the clinical trial begins. For subjects who, for any reason, cannot sign the informed consent form themselves, informed consent must be signed by their parent, legal guardian, or protector. By signing the informed consent form, the subject must also agree to allow the clinical research site to verify the original data related to the clinical research to determine the reliability of the clinical research data results.

The original signed and dated informed consent form for this trial must be securely stored by the investigator, and the signing of the informed consent form must also be recorded in the Case Report Form and relevant trial source documents.

11.4.4 **Ethics Committee and Review of Clinical Trial Documents**

The investigator is responsible for providing the clinical trial protocol, informed consent form, and information provided to subjects to the Ethics Committee to obtain independent approval for conducting this clinical research.

Ethics Committee approval must be obtained before the clinical research begins. The Ethics Committee's approval document must be sent to the investigator in writing. The approval document must include a list of all committee members who participated in the approval discussion and their respective responsibilities.

During the clinical research, any issues related to the safety of the clinical research, such as amendments to the clinical research protocol or subject informed consent form, as well as serious adverse events during the research, must be promptly reported to the Ethics Committee. The conclusion or early termination of the clinical research must also be reported to the Ethics Committee.

For subjects who suffer damage or death related to the trial (including when it cannot be determined whether the adverse event is related to the test product or diagnostic examinations required by the research protocol), treatment costs and corresponding economic compensation will be borne, except for those caused by medical accidents.

11.5 Data Handling and Record Retention

11. 5.1 Data Collection and Management

11.5.1.1 **Investigator Data Collection, Entry, and Reporting:**

Research personnel will enter information into the assessment record forms, ensuring completeness and accuracy, and instructing on-site operators to make necessary corrections or additions. If any errors requiring correction occur, modifications should be made following the assessment record form filling instructions, with the name and date of the modifier signed simultaneously.

11.5.1.2 **Database Establishment and Data Entry:** Establishing a database, data entry, and data review.

11.5.1.3 **Summary and Interim Analysis:** If data analysis is meaningful, data analysis will be conducted after the 14-day clinical observation period.

11. 5.2 Research Data Retention

All study data and source documents shall be retained for at least 5 years. Destruction before this period requires permission from the Principal Investigator.

11.6 Publication and Data Sharing Agreement

None.

11.7 Conflict of Interest Statement

None.