Union Hospital,Tongji Medical College, Huazhong University of Science and Technology
Clinical Research Project Protocol

Project Title: A clinical study evaluating the dermal safety of soluble denosumab microneedle patches

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Specialty Department: Orthopedics

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Project Summary

Study Title: A clinical study evaluating the dermal safety of soluble denosumab microneedle patches

Study Overview:

Microneedles, as a new transdermal drug delivery technology, offer advantages such as painless minimally invasive application, convenience, and safety. They have wide-ranging applications and promising development prospects. Microneedles generally refer to needles that are 10-2000 μm in height and 10-50 μm in width. The device of interest in drug delivery is the microneedle array, which consists of multiple microneedles arranged in an array on a drug carrier. Soluble denosumab microneedle patches are designed to precisely penetrate the skin's stratum corneum without reaching the pain nerve, delivering denosumab to a specific skin depth. Compared to subcutaneous injection, microneedles offer higher safety and better patient acceptance while reducing the risk of infection. Soluble microneedles overcome the limitation of traditional transdermal patches that can only deliver small molecule drugs. This has made them a hot research topic in the field of new transdermal drug delivery technologies, gaining widespread attention and recognition worldwide.

Denosumab (Denosumab) is an NF-kB receptor activator inhibitor that impacts osteoclast differentiation and development by inhibiting the OPG/RANKL-RANK bone regulatory axis. Denosumab is widely used in the treatment of bone diseases such as osteoporosis. Osteoporosis is closely related to knee osteoarthritis, where the RANKL-RANK pathway plays a key role in the pathogenesis. Therefore, we hypothesize that soluble denosumab microneedle patches may effectively treat knee osteoarthritis. Due to its good biocompatibility, polyvinylpyrrolidone (PVP) makes an excellent drug carrier platform for soluble PVP microneedles.

Study Objectives:

To investigate the skin irritation potential of soluble denosumab microneedle patches.

Study Design:

Dose escalation, single-center clinical study.

Total Number of Participants:

12 participants.

Number of Research Groups:

12 participants / 0 control group.

Group Breakdown:

12 participants (divided into 4 groups, 3 participants per group, 4 patches per participant):

- Microneedles with physiological saline
- Microneedles with 0.24 mg Denosumab
- Microneedles with 1.2 mg Denosumab
- Microneedles with 6 mg Denosumab

Diagnosis:

Osteoporosis with osteoarthritis.

Inclusion Criteria:

1. Signed and dated informed consent.
2. Commitment to comply with the study protocol and cooperate throughout the study.
3. Age 18-70, healthy or adult male or female with osteoarthritis combined with osteoporosis.
4. No history of skin diseases or other underlying conditions.
5. No antihistamines, vasodilators, vasoconstrictors, anticoagulants, hormones, or immunosuppressants within the past month.
6. If of childbearing age, women must have used contraception for at least one month before screening and commit to using contraception throughout the study period and for a specified time after the study ends.

Exclusion Criteria:

1. Pregnant or breastfeeding women.

2. Smoking >10 cigarettes per day or smoking history >20 years.
3. Presence of conditions that may interfere with the study results (e.g., severe diseases, infectious diseases, allergies, etc.) or relevant medical history.
4. Individuals receiving systemic or local treatments that affect skin homeostasis.
5. Allergic or intolerant to polyvinylpyrrolidone (PVP) or polyvinyl alcohol (PVA).
6. Contraindications for denosumab (e.g., hypocalcemia).

Study Intervention:

Denosumab, already marketed but not for this indication.

Soluble microneedle patches are not yet marketed.

Denosumab is typically administered via subcutaneous injection, whereas the soluble denosumab microneedle patch delivers denosumab encapsulated in PVP material through microneedles. Polyvinylpyrrolidone K30 (PVP-K30) is a synthetic, water-soluble polymer with excellent biocompatibility, chemical stability, and multifunctionality, widely used in biomedical fields, medical devices, and drug formulations.

Statistical Methods:

Sample size:

Primary efficacy endpoint:

Sample size: 12

Analysis: Data will be processed and analyzed using SPSS 26.0. Descriptive statistics will be provided for continuous variables using frequency counts, means ± standard deviations.

Study Duration:

July 31, 2025 - October 31, 2025.

Expected 16 weeks from recruitment of participants to data analysis, with an additional 2 weeks for follow-up.

Participant Visit Time:

3 hours.

Study Site:

Single-center in China

Tongji Medical College, Huazhong University of Science and Technology, Union Hospital

Principal Investigator Information

Principal Investigator Name, Qualifications, and Contact Information

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1.Research Background

Microneedle drug delivery technology was selected by *Scientific American*, a prestigious popular science magazine in the U.S., as one of the "Top 10 Emerging Technologies with Potential to Change Humanity" in 2020. As a novel transdermal drug delivery method, microneedles are characterized by being painless, minimally invasive, convenient, and safe. They have become a research hotspot in the field of transdermal drug delivery. Microneedles (Microneedles) consist of multiple micrometer-sized tiny needle tips arranged in an array on a base. The length of the needles ranges from 100-3000 μm, and their diameter ranges from 50-250 μm. The typical length is 250-1500 μm, depending on their specific function and application [1]. Microneedles deliver drugs by penetrating the skin's stratum corneum. Compared to traditional drug delivery methods, microneedles offer several advantages, such as avoiding the first-pass effect, reducing or eliminating pain, improving patient compliance, and enabling controlled sustained release, making them a promising technology [2].

Currently, microneedles have been extensively studied in fields such as vaccine delivery, cancer treatment, and osteoporosis drug delivery [3]. Among them, denosumab, a RANKL inhibitor, is widely used in the treatment of osteoporosis [4]. However, the traditional method of administering denosumab is subcutaneous injection, which has certain pain and infection risks. Microneedle patches, as an alternative delivery method for denosumab, have garnered attention both domestically and internationally. Research has shown that microneedle patches have higher patient acceptance and safety.

Guangzhou Xinjiyu Pharmaceutical's clinical application for "Dexmedetomidine Hydrochloride Microneedle Patches" has been successfully approved and entered the clinical trial phase. Despite this, research on the skin safety of microneedle patches is relatively limited, especially the lack of systematic clinical studies in healthy populations. Existing literature tends to focus more on the drug delivery effects of

microneedle patches, while there has been little in-depth discussion on potential skin irritation, allergic reactions, and other safety issues.

This study is designed based on the above guidelines, using the latest microneedle treatment technology—soluble denosumab microneedle patches—to explore its skin safety and clinical efficacy in treating specific diseases. Following expert consensus, the study will adhere to scientific protocols during the treatment process, ensuring patient safety and the reliability of the results.

2.Implementation of Intervention Measures

Specific Operation Methods

Before the study begins, healthy participants (meeting the inclusion criteria and excluding relevant risks) will be selected and sign an informed consent form. A total of 12 participants will be included (divided into 4 groups, 3 participants per group, 4 patches per participant):

A. Microneedle with physiological saline group
B. Microneedle with 0.24 mg denosumab group
C. Microneedle with 1.2 mg denosumab group
D. Microneedle with 6 mg denosumab group

During the intervention, 4 patches will be applied to different areas of the patella on one side of the knee joint (upper, lower, left, and right) based on the group, and the immediate and delayed skin reactions will be observed after a single intervention.

3.Study Design/Methods

3.1 Overall Overview and Key Points of Study Design

Domestic Single-Center Study

This study is designed as a dose escalation trial aimed at evaluating the safety and preliminary efficacy of different doses of denosumab (De) delivered via microneedles. The study uses a progressive grouping method with four groups, as follows:

A. Microneedle with physiological saline group (control group): 3 participants, each receiving 4 microneedles (containing only physiological saline). The 4 microneedles are applied to 4 sites on the patella of one knee joint: upper, lower, left, and right.

B. Microneedle with 0.24 mg denosumab group: 3 participants, each receiving 4 microneedles containing 0.24 mg denosumab. The 4 microneedles are applied to 4 sites on the patella of one knee joint: upper, lower, left, and right.

C. Microneedle with 1.2 mg denosumab group: 3 participants, each receiving 4 microneedles containing 1.2 mg denosumab. The 4 microneedles are applied to 4 sites on the patella of one knee joint: upper, lower, left, and right.

D. Microneedle with 6 mg denosumab group: 3 participants, each receiving 4 microneedles containing 6 mg denosumab. The 4 microneedles are applied to 4 sites on the patella of one knee joint: upper, lower, left, and right.

3.2 Study Process

The study will first complete the trial for the control group (microneedle with physiological saline) and conduct safety assessments. Once safety is confirmed, the trial for the 0.24 mg denosumab group will proceed, followed by safety evaluation. After ensuring the safety of the 1.2 mg group, the 6 mg denosumab group will be tested. Each group must complete the trial and receive clear safety results before proceeding to the next group.

3.3 Key Features

Progressive Group Design: After completing each group trial, it will be assessed against predefined criteria to determine if it can proceed to the next group.

3.4 Sample Size

This is an exploratory trial aimed at evaluating the potential effects and trends of different doses of denosumab microneedle delivery. The study is designed as a preliminary experiment, with 12 participants in total:

A. 3 participants in the microneedle with physiological saline group

B. 3 participants in the microneedle with 0.24 mg denosumab group

C. 3 participants in the microneedle with 1.2 mg denosumab group

D. 3 participants in the microneedle with 6 mg denosumab group

3.5 Safety Priority

Throughout the dose escalation process, phased assessments ensure the safety of the participants.

This design effectively minimizes potential risks associated with higher dose trials, while providing important safety and efficacy data for larger-scale future studies.

3.6 Blinding

This trial will adopt a single-blind design, where the participants will only know that the drug is delivered via microneedles, but they will not be informed of the specific dosage of denosumab in the microneedles.

3.7 Study Start and End

The study will begin after ethical review approval and the inclusion of the first participant.

The study will conclude after the last participant completes their follow-up.

Upon completion of data collection and analysis for all participants, the study will be reported to the ethical institution as completed.

4.Study Population

4.1 Diagnostic Criteria

Diagnosis based on the "Osteoarthritis Diagnosis and Treatment Guidelines" and "Osteoporosis Diagnosis and Treatment Guidelines."

4.2 Inclusion Criteria

A. Each participant must meet all inclusion criteria to be eligible for inclusion in the study:

B. Osteoporosis patients who meet the "Osteoporosis Diagnosis Criteria."

C. Age 18-70, healthy or adult male or female with osteoporosis, no drug contraindications.

D. Committed to following the study protocol and cooperating throughout the study.

E. Participant understands the related treatment process.

F. Participant has the ability to provide informed consent.

G. No use of drugs that may interfere with observation in the recent period.

4.3 Exclusion Criteria

Any baseline participant who meets one of the exclusion criteria will be excluded from the study:

A. Pregnant or breastfeeding women.

B. Smoking >10 cigarettes per day or smoking history >20 years.

C. Presence of conditions that may interfere with the study results (e.g., severe diseases, infectious diseases, allergies, etc.) or relevant medical history.

D. Individuals receiving systemic or local treatments that affect skin homeostasis.

E. Allergic or intolerant to polyvinylpyrrolidone (PVP) or polyvinyl alcohol (PVA).

F. Contraindications to denosumab, such as hypocalcemia.

4.4 Lifestyle Requirements

During the study, participants should avoid using cosmetics or skincare products that may affect skin conditions and cause infection or irritation at the intervention sites. Participants should avoid activities such as running, jumping, squatting, climbing stairs, or mountain climbing, and avoid long periods of bed rest. The application site should be kept dry, and participants should not intentionally rub or press the

application site.

Apart from the participant, at least one family member should be aware of the participant's involvement in the clinical study and be able to monitor their condition. Family members should be able to provide feedback on the participant's status during follow-up. Participants and their family members should inform the research doctor if the participant experiences severe discomfort (including the need for hospitalization).

4.5 Participant Recruitment

Study participants will be sourced from outpatient patients and healthy individuals.

Recruitment advertisements will be used for publicity.

Location: Tongji Medical College, Huazhong University of Science and Technology, Union Hospital/Orthopedics Department.

Multiple contact methods (e.g., phone, email) will be collected to ensure smooth follow-up and communication.

Participants will receive a free health assessment report after completing the study.

5.Study Objectives/Endpoints

5.1 Primary Objective (Confirmatory):

To evaluate the skin safety of soluble denosumab microneedle patches, particularly their local irritation, including erythema, edema, and allergic reactions. Also, to validate the transdermal drug delivery performance and patient acceptance, providing a safety basis for microneedle patches as a treatment for osteoporosis and knee osteoarthritis.

5.2 Secondary Objective (Exploratory):

Further evaluate the clinical potential of microneedle patches under different dosage conditions and optimize their design and dosage scheme.

5.3 Primary Outcome Measure:

a.Number of Participants With Adverse Reactions

Evaluate through skin response sheets: erythema, pain, swelling, etc. [Time Frame: Assessed at 15 minutes, 24 hours, 48 hours, and 2 weeks post-application.]

b.Amount of pain

Pain will be measured using the faces pain scales revised (0-2-4-6-8-10, 0: no pain, 10: very much pain). [Time Frame: Assessed at 15 minutes, 24 hours, 48 hours, and 2 weeks post-application.]

c.Number of injection site reactions

Injection site assessment for the following: Pain (grade 1/2/3) Tenderness (grade 1/2/3) Pruritus (grade 1/2/3) Erythema (absent/present) Induration (grade 1/2/3) Blister (absent/present) Ulceration (absent/present) Necrosis (absent/present) Ecchymosis (absent/present) If any of these signs or symptoms is present, it is regarded an injection site reaction. [Time Frame: Assessed at 15 minutes, 24 hours, 48 hours, and 2 weeks post-application.]

5.4 Secondary Outcome Measures:

d.Soluble Denosumab Microneedle Patch Subject Satisfaction Questionnaire

1. How satisfied are you with the application process of the microneedle patch?
    5 = Very Satisfied
    4 = Satisfied
    3 = Neutral
    2 = Dissatisfied
    1 = Very Dissatisfied
2. How satisfied are you with the overall skin condition around the knee after using the microneedle patch?
    5 = Very Satisfied
    4 = Satisfied
    3 = Neutral
    2 = Dissatisfied
    1 = Very Dissatisfied
3. Overall, how satisfied are you with the ease of using the microneedle patch?

5 = Very Satisfied

4 = Satisfied

3 = Neutral

2 = Dissatisfied

1 = Very Dissatisfied

4. Overall, now that you have tried this microneedle patch and completed the study, how likely are you to use this treatment again in the future?

5 = Very Likely

4 = Somewhat Likely

3 = Neutral

2 = Somewhat Unlikely

1 = Very Unlikely

5. Overall, how likely are you to recommend this microneedle patch treatment to a family member or friend?

5 = Very Likely

4 = Somewhat Likely

3 = Neutral

2 = Somewhat Unlikely

1 = Very Unlikely

[Time Frame: Assessed at 2 weeks post-application.]

e. Number of participants with abnormal laboratory tests results

Evaluation of blood routine and blood electrolytes to monitor participants' health status and safety. [Time Frame: Assessed at 2 weeks post-application.]

5.5 Other Pre-specified Outcome Measures:

f. Frequency of both local and systemic Adverse Events

An adverse event is any adverse change from the subject's baseline condition, i.e. any subjective signs and symptoms, or change in a concomitant disease present at the screening visit. This includes inter-current signs, symptoms, illness and significant deviations from baseline which may occur during the course of the clinical study, whether considered related to treatment or not. [Time Frame: 2 weeks]

6.Data Collection, Management, and Statistical Methods

6.1 Data Collection Methods

Data collection will be conducted by clinical researchers under the supervision of the principal investigator, who will be responsible for the accuracy, completeness, and timeliness of the reported data. All data must be clearly recorded to ensure traceability.

A secure database will be established, protected by password, with logical validation programs set up during database creation.

6.2 Data Management

Data entry can be done through double data entry, meaning two different researchers will enter the same data, followed by comparison of the results to ensure accuracy. Range checks will be set to ensure the entered data fall within the expected reasonable range (e.g., certain variables should fall within physiological or experimental expected ranges). Uniform coding rules will be applied to sensitive data, such as encrypting participant names, ID numbers, etc., to avoid directly exposing sensitive information.

Data will be stored in a secure database system with restricted access, allowing only authorized personnel to access it.

The data entry interface will include automatic checks for logical errors and range errors.

If imaging data or other materials are to be retained after the study, participant consent will be obtained through the informed consent form before the study begins, and it will be clarified that such materials may be used for future related research.

All study data and original documents will be kept for a minimum of 2 years

before destruction, with permission required before disposal.

6.3 Statistical Methods

For descriptive statistics, percentages will be used for categorical data, and mean ± standard deviation will be used for continuous data.

For inferential statistics, p-values and confidence intervals will be calculated, specifying one-tailed or two-tailed tests.

No prior hypothesis testing for covariates (e.g., normality tests) is required, and no transformations or non-parametric tests will be performed. The primary outcome variable is the incidence rate of adverse reactions in patients, analyzed using independent samples t-test and chi-square tests.

The secondary outcome variables include VAS pain scores and knee joint OKS functional scores, analyzed using independent samples t-test.

Mean, non-adherence, and lost-to-follow-up data will be described using mean ± standard deviation, percentages, and 95% confidence intervals.

Missing data will not be included in the analysis.

7.References

[1] PETTIS R J, HARVEY A J. Microneedle delivery: clinical studies and emerging medical applications[J]. Ther Deliv, 2012, 3(3):357-71.

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[4] SOBACCHI C, FRATTINI A, GUERRINI M M, et al. Osteoclast-poor human osteopetrosis due to mutations in the gene encoding RANKL[J]. Nat Genet, 2007, 39(8):960-2.