

Clinical Safety Evaluation and Preliminary Efficacy Study of Subcutaneous Myografts Transplantation

Study Protocol

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Background

With the acceleration of population aging and the increasing burden of chronic diseases, skeletal muscle atrophy and functional decline in patients with long-term bed rest have become major clinical challenges that require urgent attention(1). Prolonged bed rest leads to muscle disuse, reduced protein synthesis, and rapid loss of muscle fibers, resulting in decreased muscle strength, reduced exercise tolerance, and metabolic dysfunction. More importantly, skeletal muscle is not only the primary organ responsible for movement but is also recognized as a critical endocrine organ. Through the secretion of multiple myokines, skeletal muscle regulates systemic metabolism, inflammatory responses, and the function of various organs. When skeletal muscle atrophy occurs, the secretion of myokines with anti-inflammatory, metabolic regulatory, and multi-organ protective effects is markedly reduced. This loss of endocrine function further exacerbates insulin resistance, chronic inflammation, and multisystem degenerative changes. Current rehabilitation strategies, including passive exercise, physical therapy, and nutritional interventions, show limited efficacy in preventing muscle atrophy or improving muscle function in patients with prolonged immobilization. Moreover, these approaches are insufficient to maintain long-term muscle mass and function. Therefore, there is an urgent need to develop innovative intervention strategies that can effectively reverse or delay muscle atrophy and functional deterioration in patients with long-term bed rest, thereby reducing complication risk, improving quality of life, and extending health span.

In recent years, cell-based therapies have gained increasing attention. Muscle stem cells (MuSCs) are the source cells responsible for skeletal muscle regeneration and possess the capacity for self-renewal and differentiation muscle injury, MuSCs proliferate as myoblasts, differentiate into myocytes, and ultimately fuse to form multinucleated muscle fibers. Myoblast transplantation has been explored as a therapeutic approach for muscle atrophy and other muscle-related disorders and can also

serve as a delivery platform for therapeutic proteins. However, a major limitation of myoblast transplantation is the poor survival of transplanted cells, with most cells dying within 48 hours after transplantation due to local ischemia, apoptosis, inflammation, or immune rejection. In contrast, mature myocytes represent terminally differentiated cells and provide a safe, efficient, and programmable cell therapy platform. In addition to their potential for local tissue repair, mature myocytes also show promise as long-term delivery vehicles for therapeutic proteins. Compared with other cell types, mature myocytes exhibit distinct advantages in terms of safety and biological function. On the one hand, muscle fiber growth primarily relies on multinucleated hypertrophy rather than cell division, thereby minimizing tumorigenic risk(5-8). On the other hand, mature myocytes possess strong angiogenic potential, which facilitates vascular reconstruction and long-term graft survival after transplantation. To address these clinical limitations, we previously developed a novel technique for subcutaneous transplantation of autologous differentiated myocytes, generating engineered muscle grafts with spontaneous contractile activity and favorable vascularization. In preclinical animal studies, these grafts demonstrated long-term survival in vivo and mimicked a state of "continuous exercise." In addition, they stably secreted myokines and systemically improved muscle quality, bone mineral density, energy metabolism, and inflammatory status. These effects were consistently observed in both aging mouse models and obesity models, with significant attenuation of degenerative changes.

In humans, application of this autologous differentiated myocyte subcutaneous transplantation technique has the potential to overcome key limitations of conventional myocyte transplantation, including low graft survival and inadequate vascularization. By enabling long-term graft survival and functional maintenance, and through spontaneous sustained contraction and stable secretion of multiple myokines, this strategy may fundamentally improve skeletal muscle atrophy, sarcopenia, and systemic metabolic disorders in patients with long-term bed rest. The present study aims to translate autologous differentiated myocyte subcutaneous transplantation into clinical application in patients with muscle atrophy caused by long-term bed rest, and to explore its safety, feasibility, and preliminary efficacy. This study is intended to provide a solid foundation for the clinical translation of autologous differentiated myocyte subcutaneous transplantation as a therapeutic strategy.

Reference:

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Study Objectives

At present, there is a lack of effective clinical strategies for patients with muscle atrophy caused by long-term bed rest due to multiple underlying conditions, to reverse or delay muscle atrophy and functional degeneration. Conventional rehabilitation training and nutritional support have limited efficacy in mitigating muscle atrophy induced by prolonged immobilization, highlighting the urgent need for novel therapeutic interventions. Based on our previous experimental findings, we have developed an autologous differentiated myocyte subcutaneous transplantation technique that enables long-term graft survival in vivo, mimics a state of "continuous exercise," and ensures stable secretion of myokines. Through these mechanisms, this approach systemically improves muscle quality, bone mineral density, energy metabolism, and inflammatory status. Building on these preclinical results, the present study aims to innovatively translate autologous differentiated myocyte subcutaneous transplantation into human application, with the goal of constructing a sustainable, spontaneously contractile, and endocrine-functional "muscle graft."

The specific objectives of this study are as follows: (1) To evaluate graft survival, vascularization, and immune tolerance following subcutaneous transplantation of

autologous differentiated myocytes in patients with long-term bed rest – related muscle atrophy, and to ensure the safety of clinical application. (2) To systematically assess the effects of the muscle graft on skeletal muscle mass, muscle strength, and exercise endurance, and to explore its potential in reversing muscle atrophy and preserving muscle function. (3) To analyze the regulatory effects of graft-derived myokines on systemic energy metabolism, bone mineral density, and chronic inflammatory status, and to evaluate their impact on aging-related degenerative conditions, including sarcopenia, osteoporosis, and metabolic disorders.

Ultimately, this study aims to establish a subcutaneous transplantation strategy for autologous differentiated myocytes capable of generating long-term surviving, vascularized, and spontaneously contractile muscle grafts, and to explore further the therapeutic potential of mimicking the skeletal muscle secretory profile during physical activity for the treatment of muscle atrophy, sarcopenic obesity, and aging-related degenerative diseases.

Study Content

This study focuses on the safety and functional validation of autologous subcutaneous transplantation of differentiated myocytes. The main study components include muscle cell sourcing and engineering, subcutaneous implantation and long-term observation, safety evaluation, and functional assessment using disease-relevant outcome measures. (1) Muscle Cell Sourcing and Engineering: Recruited participants will undergo muscle biopsy (e.g., from the vastus lateralis or gluteus medius) to obtain a small amount of skeletal muscle tissue. Muscle satellite cells will be isolated, purified, expanded in vitro, and induced to differentiate into mature myocytes. Quality control of the differentiated myocytes will be performed, including assessments of cell purity, functional characteristics, and secretory profiles, to ensure suitability for clinical transplantation. (2) Subcutaneous Implantation and Long-Term Observation: Engineered myocytes will be mixed with collagen and injected subcutaneously into the abdominal region. Dynamic evaluations will be conducted to assess graft survival, vascular reconstruction, contractile function, and local tissue responses. Electromyography and imaging modalities (MRI and ultrasonography) will be used to monitor graft contractility and tissue integration. (3) Safety Evaluation: Local and systemic safety will be assessed

through continuous monitoring. Serum immunological parameters, including white blood cell counts and inflammatory cytokines, will be measured. Local histopathological examination will be performed using graft tissue biopsy when indicated. Ectopic transplantation, cell migration, abnormal differentiation, or pathological proliferation will be actively excluded. Adverse events will be recorded during long-term follow-up. (4) Functional Assessment: Functional outcomes will include measurements of skeletal muscle mass using dual-energy X-ray absorptiometry (DXA), bone metabolism indicators, serum creatine kinase levels, and systemic energy metabolism parameters, including respiratory exchange ratio (RER) and energy expenditure.

Study Design

1. Study Design and Control Strategy

- (1) Study Type: Prospective, single-arm, self-controlled pre–post interventional study.
- (2) Control Strategy: Within-subject control: Each participant will complete the same set of assessments before and after the intervention, serving as their own control. A within-subject pre – post comparison will be used to evaluate individualized responses and temporal changes induced by subcutaneous muscle graft transplantation.

2. Sample Size

- (1) Total Sample Size: 6 participants.
- (2) This study is designed as a Phase I/IIa exploratory clinical trial. According to common practice in early-phase cell therapy studies, including CAR-T therapy, myoblast transplantation, and induced pluripotent stem cell (iPSC) – based interventions, an initial cohort of 3 – 6 participants per group is typically enrolled for preliminary safety and feasibility evaluation. Given that the primary objectives of this study are to assess the safety, feasibility, and preliminary efficacy of autologous differentiated myocyte subcutaneous transplantation in patients with long-term bed rest – induced muscle atrophy, a small-sample design with intensive follow-up has been adopted. This approach allows close monitoring of adverse events and biological responses while generating foundational data to support subsequent larger-scale clinical studies.

3. Study Population and Grouping

(1) Inclusion Criteria:

- 1) Age: 18–80 years, with no restriction on sex.
- 2) Long-Term Bed Rest: Continuous bed rest for ≥ 4 weeks. Causes may include neurological injuries (such as brain death, stroke, or spinal cord injury), recovery after major orthopedic surgery, intensive care unit stay, or activity limitation due to chronic diseases.
- 3) Evidence of Muscle Atrophy: A significant reduction in muscle mass or muscle strength confirmed by clinical assessment and imaging studies. According to dual-energy X-ray absorptiometry (DXA), appendicular skeletal muscle mass index ($\text{ASM}/\text{height}^2$) $< 7.0 \text{ kg}/\text{m}^2$ in men and $< 5.4 \text{ kg}/\text{m}^2$ in women, in accordance with the EWGSOP2 criteria.
- 4) Stable Underlying Conditions: Underlying medical conditions are stable, with no acute exacerbation, and an APACHE II score between 0 and 20.
- 5) Surgical Eligibility: Absence of severe comorbidities that contraindicate surgical or transplantation interventions.
- 6) Informed Consent: Written informed consent obtained from the participant, an immediate family member, or a legal guardian, agreeing to undergo muscle biopsy. General health status must permit subcutaneous transplantation.

(2) Exclusion Criteria

- 1) History of malignant tumors.
- 2) Coagulation disorders or current use of anticoagulant therapy.
- 3) Active infection or immunodeficiency.
- 4) Severe cardiac or renal insufficiency (estimated glomerular filtration rate $< 60 \text{ mL}/\text{min}/1.73 \text{ m}^2$; New York Heart Association class III – IV heart failure).
- 5) Muscle-related diseases, including hereditary myopathies (such as muscular dystrophy) or acquired myositis (creatinine kinase > 3 times the upper limit of normal).
- 6) Severe local skin lesions or a history of allergic reactions at the injection site.
- 7) Use of muscle growth – modulating medications (such as testosterone or growth

hormone) within the past 6 months.

- 8) Long-term use of corticosteroids or immunosuppressive therapy, including anti-rejection medications following organ transplantation.
- 9) Participation in other interventional clinical trials (excluding observational studies).
- 10) Any other medical or ethical conditions deemed by the investigator to make the participant unsuitable for enrollment.

(3) Study Grouping:

- 1) Intervention Group: Participants will receive subcutaneous transplantation of autologous differentiated myocytes.

4. Intervention Procedures and Standardized Workflow

(1) Principles of Study Design

This study adopts a strategy of subcutaneous transplantation of autologous differentiated myocytes. Muscle satellite cells are isolated from autologous skeletal muscle tissue, expanded and induced to differentiate into myocytes in vitro, and combined with collagen to form an engineered muscle graft (myograft). The graft is then implanted via subcutaneous injection. The study aims to evaluate long-term graft survival, spontaneous contractile activity, and myokine secretion capacity in humans, and to explore the potential clinical value of this approach in enhancing muscle function, regulating metabolic status, and delaying aging-related degenerative changes. This study is intended to provide a technical foundation for the translational application of skeletal muscle – based cell therapy in patients with long-term bed rest – induced muscle atrophy and chronic diseases.

(2) Timeline of Sample Collection and Intervention Procedures

1) Preoperative Preparation and Assessment

Before intervention, all participants will undergo a comprehensive physical examination and laboratory testing, including complete blood count, serum biochemistry, liver and renal function tests, infection screening, and coagulation assessment, to reconfirm eligibility and exclude contraindications. The study team will explain the study protocol in detail, and written informed consent will be obtained. The muscle biopsy site will be determined in advance, with the vastus lateralis selected as the preferred site.

Preoperative marking and scheduling will be completed accordingly.

2) Timeline of Sample Collection and Intervention Procedures

Procedure Setting: Muscle biopsy will be performed under sterile conditions in a minor procedure room or outpatient surgical suite.

Anesthesia: Local anesthesia with 1% lidocaine will be administered to the skin and underlying fascia.

Biopsy Technique: A Bergström biopsy needle will be used to obtain skeletal muscle tissue through a 0.5 – 1.0 cm skin incision. Approximately $0.5 \times 0.5 \times 0.5 - 1.0$ cm of muscle tissue will be collected in a single attempt whenever possible, minimizing fascia contamination and avoiding repeated needle insertions.

Postoperative Care: After the procedure, a compression dressing will be applied and covered with a sterile dressing. Participants may leave after 30 minutes of observation if no discomfort occurs. Strenuous activity, local compression, and washing of the biopsy site will be avoided within 24 hours.

3) Cell Preparation and Injection Readiness

Muscle tissue will be immediately transferred to a GMP-compliant laboratory for isolation, expansion, and differentiation of muscle satellite cells into myocytes, a process requiring approximately 10 – 14 days. All cell products must pass quality control assessments, including sterility testing, cell viability, and differentiation efficiency, before being approved for clinical use. Prepared cell products will be packaged by designated personnel and transported under cold-chain conditions to the injection site. Participant identity and batch codes will be verified again prior to injection. On the day of injection, each participant will receive a total dose of 1×10^8 cells, mixed with 6 mL of collagen.

4) Subcutaneous Injection of Muscle Grafts

Injection Site: The abdominal subcutaneous region will be selected preferentially, avoiding bony prominences and areas with dense vascular distribution. **Patient Position:** Participants will be placed in a prone or seated position. **Anesthesia:** Local subcutaneous anesthesia with 1% lidocaine.

Injection Procedure:

- I. Bilateral, multi-point injections will be performed.

- II. Injection volume per side: 2.5 – 3.0 mL.
- III. Maximum volume per injection point: ≤ 1.0 mL.
- IV. A 21-G needle will be used to create subcutaneous channels.
- V. A total of 1×10^8 cells will be injected bilaterally at multiple sites, with an injection depth of 1–2 cm.
- VI. The graft will be distributed in a planar pattern rather than as a compact mass to facilitate integration.
- VII. After injection, the graft will be allowed to solidify for 5 – 10 minutes.

Hemostasis will be achieved by compression, followed by sterile dressing and pressure bandaging. Injection parameters, including batch number, time, operator, and injection sites, will be recorded. Participants will be observed for one hour post-procedure and discharged if no discomfort is noted. Strenuous activity and washing of the injection area will be avoided for 72 hours.

5) Postoperative Observation and Follow-Up

Immediate Observation (Within 24 Hours): Vital signs, including body temperature, blood pressure, and heart rate, will be closely monitored. Signs of allergic reactions or acute immune responses will be assessed.

Short-Term Follow-Up: At Day 1, Day 7, and Day 14 post-injection, the injection site will be evaluated for redness, swelling, induration, and nodules. Complete blood count and C-reactive protein (CRP) levels will be measured to assess acute inflammation.

Mid-Term Follow-Up: Preliminary therapeutic effects will be evaluated. Graft survival will be assessed using imaging modalities. Laboratory assessments will include complete blood count, CRP, inflammatory cytokines, liver enzymes, creatine kinase, DXA-derived ASM, muscle ultrasonography, myokines, bone mineral density, bone turnover markers, and metabolic parameters assessed by indirect calorimetry.

Long-Term Follow-Up: Participants will be followed for up to 12 months. Long-term endpoints will include skeletal muscle mass (DXA), bone mineral density, serum calcium and phosphorus, metabolic indicators, and muscle enzyme profiles.

Management of Abnormal Findings: Local redness, pain, or mild enzyme elevation will be recorded and observed. Persistent induration, abnormal growth, or redness lasting

more than one week will prompt ultrasonography or MRI, and biopsy will be performed if necessary to exclude ectopic growth or abnormal proliferation.

Adverse Event Management: Any serious adverse events, including high fever, widespread rash, respiratory distress, allergic reactions, or marked elevation of muscle enzymes, will be immediately reported to the research team and the ethics committee.

5. Outcome Measures

(1) Primary Outcome Measures

- 1) **Vital Signs Monitoring:** Measurement of blood pressure, heart rate, respiratory rate, and body temperature.
- 2) **Local Adverse Reactions:** Visual inspection of local reactions, including redness, swelling, induration, and infection at the injection site.
- 3) **Evaluation of Myocyte Graft Survival:** Gross visual assessment of changes in graft volume. Ultrasonographic evaluation of graft morphology, echo uniformity, and the presence of necrosis or fluid accumulation. Assessment of blood perfusion to determine vascular regeneration and graft vascularization.
- 4) **Inflammatory and Immune Status:** Complete blood count (CBC): total white blood cell count and differential percentages, with calculation of the neutrophil-to-lymphocyte ratio (NLR). Inflammatory cytokines: IL-6, TNF- α , IFN- γ , and IL-1 β , quantified by enzyme-linked immunosorbent assay (ELISA). C-reactive protein (CRP): assessment of systemic inflammatory status. Anti-inflammatory cytokine: IL-10, reflecting immune regulatory status.
- 5) **Liver Enzymes and Metabolic Safety:** Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) will be analyzed to assess metabolic safety of the intervention.
- 6) **Skeletal Muscle Mass (DXA Assessment):** Appendicular skeletal muscle mass (ASM) will be measured using dual-energy X-ray absorptiometry (DXA), and the skeletal muscle mass index (ASMI, kg/m²) will be calculated.
- 7) **Muscle Thickness (Muscle Ultrasonography):** High-frequency ultrasonography will be used to measure the thickness of representative muscle groups (e.g., rectus femoris), defined as the distance from skin to periosteum. Each site will be measured

three times, and the mean value will be recorded.

- 8) Circulating Myokines: Serum levels of FGF21, irisin, IGF-1, and BDNF will be measured to evaluate myokine secretion from the muscle graft.

(2) Secondary Outcome Measures

- 1) Bone mineral density of the lumbar spine and femur will be assessed using DXA or quantitative computed tomography (QCT). T-scores and trabecular microarchitecture parameters will be analyzed to evaluate improvements in bone metabolism.
- 2) Bone Turnover Markers: Serum levels of procollagen type I N-terminal propeptide (P1NP), C-terminal telopeptide of type I collagen (CTX), and alkaline phosphatase (ALP) will be measured to assess bone formation, bone resorption, and overall bone metabolic activity.
- 3) Lipid Profile: Serum triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) will be measured to evaluate lipid metabolism and cardiovascular risk.
- 4) Glucose Metabolism and Insulin Resistance: Fasting blood glucose (FBG) will be measured using venous blood samples. Fasting insulin (FINS) levels will also be assessed, and insulin resistance will be evaluated using the homeostasis model assessment of insulin resistance ($\text{HOMA-IR} = \text{FBG} \times \text{FINS} / 22.5$).
- 5) Energy Expenditure: Energy metabolism will be assessed using indirect calorimetry to measure the respiratory exchange ratio (RER) and resting metabolic rate (RMR).
- 6) Body Fat Assessment: Whole-body DXA scanning will be performed to evaluate total body fat mass and fat distribution.

6. Safety and Efficacy Evaluation

(1) Safety Evaluation

Safety assessment in this study focuses on the standardized implementation and ethical compliance of autologous differentiated myocyte preparation, subcutaneous transplantation procedures, and post-intervention follow-up. The main components include the following:

- 1) **Adverse Event Monitoring:** All participants will undergo systematic safety evaluations before, during, and after the intervention. Local complications related to subcutaneous transplantation (such as redness, swelling, induration, infection, or abnormal tissue growth), rare systemic adverse reactions (such as allergic reactions, high fever, or elevated creatine kinase levels), and serious adverse events (SAEs) requiring hospitalization, surgical intervention, or meeting criteria for study termination will be recorded. All adverse events will be graded according to the Common Terminology Criteria for Adverse Events (CTCAE), version 5.0, with reference to established safety benchmarks from international cell therapy studies
- 2) **Ethical Compliance:** All participants will voluntarily sign written informed consent forms approved by an independent ethics committee. The target informed consent signing rate is 100%. The study protocol will be conducted in strict accordance with ethical standards and regulatory requirements.
- 3) **Data and Sample Privacy Protection:** All biological samples will be managed using de-identified coding, with unique anonymous identifiers replacing personal information. Clinical data and biological samples will be stored separately with controlled access. The collection, storage, and analysis of samples and clinical data will strictly comply with relevant national regulations, including the Personal Information Protection Law of the People's Republic of China and the Regulations on the Administration of Human Genetic Resources.

(2) Efficacy Evaluation

The therapeutic effects of autologous differentiated myocyte graft transplantation will be comprehensively evaluated across three dimensions: functional improvement, biomarker validation, and systemic metabolic and inflammatory modulation.

- 1) **Functional Outcomes:** Improvements in skeletal muscle mass (ASMI) and handgrip

strength (in participants with preserved upper limb function) will be used as primary clinical efficacy indicators. An intervention will be considered effective if, at 3 and/or 6 months post-intervention, these indicators show a relative improvement of $\geq 10\%$ or recovery beyond the diagnostic thresholds defined by the Asian Working Group for Sarcopenia (AWGS).

- 2) **Biomarker Validation:** Muscle-derived biomarkers, including MyoG, Pax7, and Myh1/4, will be assessed at predefined time points after intervention. Statistically significant changes compared with baseline and reference values will be used to support biological efficacy.
- 3) **Metabolic and Inflammatory Improvement:** Within-subject pre – post comparisons will be conducted to evaluate changes in metabolic indicators, including resting metabolic rate (RMR), respiratory exchange ratio (RER), body fat proportion (DXA), and insulin resistance index (HOMA-IR). Concurrently, reductions in pro-inflammatory markers (CRP, IL-6, TNF- α) and increases in the anti-inflammatory cytokine IL-10 will be evaluated as indicators of systemic immune modulation.

7. Statistical Analysis and Quality Control

(1) Statistical Analysis

- 1) Between-group differences will be analyzed according to data type and distribution. Comparisons between two groups will be performed using the independent-samples t test for normally distributed data or the Mann – Whitney U test for non-normally distributed data. Categorical variables will be compared using the chi-square test or Fisher's exact test. Paired pre – post intervention data will be analyzed using the paired t-test or the Wilcoxon signed-rank test.
- 2) Longitudinal follow-up data collected at multiple time points will be analyzed using repeated-measures analysis of variance (RM-ANOVA) or linear mixed-effects models (LMMs) to account for time effects and intervention effects.
- 3) For analyses involving multiple biomarkers, such as inflammatory cytokines, false discovery rate (FDR) correction will be applied using the Benjamini – Hochberg method. Spearman or Pearson correlation analyses will be conducted to explore associations between these biomarkers and clinical outcomes.

- 4) All statistical tests will be two-sided. A P value < 0.05 will be considered statistically significant, and $P < 0.01$ will be considered highly significant.

(2) Quality Control

- 1) Standardization of data collection: All clinical measurements will be performed using standardized equipment and operating procedures (SOPs). Personnel will be trained before measurements. Key endpoint assessments will be conducted by the same testing center and, whenever possible, by the same personnel to ensure batch consistency.
- 2) Double data entry and verification: All paper case report forms (CRFs) will be independently entered by two researchers and cross-checked. Built-in logical validation procedures will automatically flag discrepancies to ensure data accuracy.
- 3) Participant compliance management: Dedicated follow-up records will be established to ensure timely sample collection and data completeness. At each follow-up visit, physicians will confirm completion of the intervention. Reminder messages and manual follow-up calls will be implemented as needed.
- 4) Sample and data traceability system: Biological samples will be labeled with anonymous identifiers and stored separately from personal identifying information. The entire process of sample transport, processing, and cryopreservation will be fully documented, including time points, personnel, and conditions, to ensure closed-loop traceability.
- 5) Adverse event documentation and review: All adverse events (AEs) and serious adverse events (SAEs) will be recorded in dedicated AE/SAE forms, graded, and signed by the principal investigator. SAEs will be reported to the ethics committee and monitoring authorities within 24 hours, and expert evaluation procedures will be initiated promptly.

Eligibility Criteria, Withdrawal, and Study Termination Criteria

1. Inclusion Criteria

(1) General Conditions:

- 1) Written informed consent obtained from the participant's immediate family member

or legal guardian, agreeing to undergo muscle biopsy.

- 2) General health status adequate to permit subcutaneous transplantation procedures.

(2) Patients With Long-Term Bed Rest–Induced Muscle Atrophy

- 1) Age 18 – 80 years, with no restriction on sex.
- 2) History of long-term bed rest: continuous bed rest for ≥ 4 weeks, with causes including neurological injury (such as brain death, stroke, or spinal cord injury), recovery after major orthopedic surgery, intensive care unit stay, or activity limitation due to chronic diseases.
- 3) Evidence of muscle atrophy: a marked reduction in muscle volume or muscle strength confirmed by clinical assessment and imaging studies. According to dual-energy X-ray absorptiometry (DXA), appendicular skeletal muscle mass index (ASM/height²) $< 7.0 \text{ kg/m}^2$ in men and $< 5.4 \text{ kg/m}^2$ in women, based on EWGSOP2 criteria.
- 4) Written informed consent obtained from the participant's immediate family member or legal guardian, agreeing to undergo muscle biopsy, with general health status adequate to permit subcutaneous transplantation.
- 5) Stable underlying medical conditions (APACHE II score 0 – 20), with no acute exacerbation or severe comorbidities that would contraindicate surgical or transplantation interventions.

2. Exclusion Criteria

- 1) History of malignant tumors.
- 2) Coagulation disorders or current use of anticoagulant therapy.
- 3) Active infection or immunodeficiency.
- 4) Severe cardiac or renal insufficiency (estimated glomerular filtration rate $< 60 \text{ mL/min/1.73 m}^2$; New York Heart Association [NYHA] class III – IV heart failure).
- 5) Muscle-related diseases, including hereditary myopathies (such as muscular dystrophy) or acquired myositis (creatinine kinase > 3 times the upper limit of normal).
- 6) Severe local skin lesions or a history of allergic reactions at the injection site.
- 7) Use of muscle growth – modulating medications (such as testosterone or growth

hormone) within the past 6 months.

- 8) Long-term use of corticosteroids or immunosuppressive therapy, including anti-rejection medications following organ transplantation.
- 9) Other exclusion criteria: participation in other interventional clinical trials (excluding observational studies).
- 10) Any other medical or ethical conditions deemed by the investigator to make the participant unsuitable for enrollment.

3. Withdrawal and Study Termination Criteria

(1) Participant or Family-Initiated Withdrawal

- 1) Withdrawal of informed consent at any time.
- 2) Inability to continue follow-up due to participant- or family-related reasons (e.g., relocation or scheduling conflicts).

(2) Study Termination Criteria

- 1) Medical reasons: occurrence of serious adverse events (SAEs), such as uncontrollable bleeding requiring surgical intervention after biopsy; newly identified contraindications (e.g., diagnosis of malignant tumor after enrollment); failure to obtain an adequate muscle tissue sample during biopsy; or inability to culture and obtain enough transplantable cells.
- 2) Protocol deviations: failure to process samples according to standardized procedures or discovery that the participant did not meet inclusion criteria at enrollment.
- 3) Investigator decision: occurrence of severe safety events beyond acceptable control related to the intervention, or termination required by the ethics committee.

Management Plan for Common Adverse Events and Serious Adverse Events

1. Definition and Grading of Adverse Events

- 1) Common adverse events (AEs): Grade 1 – 2 according to CTCAE version 5.0, including local pain, mild bleeding, and transient fever ($\leq 38.5^{\circ}\text{C}$).

- 2) Serious adverse events (SAEs): Grade ≥ 3 according to CTCAE version 5.0, including infections requiring hospitalization, anaphylactic shock, deep vein thrombosis, and other severe conditions.

2. Management Plan for Common Adverse Events

Type of Adverse Event	Management Measures	Responsible Personnel
Local skin reactions	1. Assess pain intensity (VAS score);2. Oral nonsteroidal anti-inflammatory drugs (ibuprofen 400 mg, q8h, ≤ 3 days);3. Local cold compress (15 min per session, every 2 hours).	Surgeon
Local hematoma	1. Compression bandaging (elastic bandage for 24 hours);2. Ultrasonographic evaluation of hematoma extent (if diameter >5 cm);3. Discontinue anticoagulant medications until hematoma resolution.	Surgeon
Superficial infection	1. Bacterial culture and antimicrobial susceptibility testing;2. Empirical oral cefuroxime axetil 500 mg twice daily (in patients without allergy);3. Daily wound dressing until healing.	Infectious disease consultation
Low-grade fever (≤ 38.5 °C)	1. Complete blood count and CRP testing;2. Physical cooling (tepid sponging);3. Monitor body temperature every 4 hours for 48 hours.	Surgeon
Mild allergic reaction (rash/pruritus)	1. Discontinue suspected agents or materials;2. Oral antihistamines (e.g., loratadine 10 mg once daily for 3 days);3. Continuous observation of symptom progression.	Surgeon
Injection-site induration	1. Evaluate the nature of induration; 2. Local warm compress to promote absorption; 3. Weekly reassessment; ultrasonography if necessary.	Surgeon

3. Management Plan for Serious Adverse Events

Type of SAE	Emergency Management Procedures	Reporting Timeline
Active bleeding	1. Immediate local compression for hemostasis (≥ 15 min);2. Intravenous tranexamic acid 1 g (within 30 min);3. Vascular interventional hemostasis is ineffective.	Immediate (report to ethics committee within one hour)
Anaphylactic shock	1. Intramuscular epinephrine 0.3 mg (lateral thigh);2. Establish intravenous access (rapid infusion of 500 mL normal saline);3. Cardiac monitoring and transfer to the ICU.	Immediate (activate in-hospital emergency code)
Deep vein thrombosis (DVT)	1. Confirm diagnosis by lower-limb vascular ultrasonography;2. Low-molecular-weight heparin (enoxaparin 1 mg/kg q12h);3. Avoid massage of the affected limb.	Within 24 hours
Sepsis	1. Blood cultures and broad-spectrum antibiotics (meropenem 1 g q8h);2. Fluid resuscitation (30 mL/kg crystalloid);3. Transfer to infectious disease intensive care.	Immediate (written report within 6 hours)
Abnormal growth	1. Ultrasonography or soft-tissue MRI to define structure;2. If abnormal nodular structure, active proliferation, or recurrent growth is observed, classify as an adverse outcome endpoint;3. Surgical excision with immunohistochemistry and genetic testing to exclude tumorigenic risk.	Immediate (written report within 6 hours)
Muscle necrosis/rhabdomyolysis	1. Discontinue intervention;2. Intravenous fluid resuscitation (0.9% saline or lactated Ringer's solution) to maintain urine output ≥ 200 mL/h;3. Monitor renal function to prevent acute kidney injury (AKI).	Immediate (written report within 6 hours)
Autoimmune response activation	1. Rheumatology consultation;2. Serological testing;3. Evaluate the need for immunomodulatory intervention.	Within 24 hours

Type of SAE	Emergency Management Procedures	Reporting Timeline
Multifocal injection-site infection	1. Culture and broad-spectrum antibiotics (meropenem 1 g q8h);2. Debridement and culture;3. Evaluate suspension of subsequent injections.	Immediate (written report within 6 hours)

4. Insurance of Plan Implementation

(1) Emergency Resource Allocation: Emergency kits will be readily available in the biopsy procedure room, including epinephrine, tranexamic acid, and sterile dressings. A 24-hour on-call emergency response team will be maintained.

(2) Personnel Training: Quarterly simulation drills for emergency scenarios (e.g., bleeding and allergic reactions). All study personnel will hold valid Basic Life Support (BLS) certification.)

(3) Process Coordination: Upon occurrence of an SAE, the principal investigator will complete the Serious Adverse Event Report Form and simultaneously submit it to the ethics committee and regulatory authorities. Follow-up reports will be submitted every 72 hours until the event is resolved or stabilized.

5. Documentation and Traceability

(1) AE/SAE documentation: Records will include time of occurrence, management measures, and outcomes (recovery, sequelae, or death).

(2) Sample-related causality analysis: If an SAE is deemed possibly or more likely related to the study procedures, similar interventions will be suspended until completion of a root cause analysis (RCA).

(3) Ethical archiving: All SAE-related documents will be retained for five years after study completion. AE/SAE.