Tibial Cortex Transverse Transport Accelerates Diabetic Foot Ulcer Healing via Enhanced Mesenchymal Stem Cells Mobilization and Homing

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INTRODUCTION: Diabetic foot ulcer (DFU) is a severe complication of diabetes that carries a higher risk of amputation and mortality. Mesenchymal stem cells (MSCs) dysfunction in diabetes mellitus may play an essential role in the pathogenesis of DFU. However, current treatment methods for severe DFU are unsatisfactory. Distraction osteogenesis (DO) can induce histogenesis through multiple mechanisms, including promoting MSCs mobilization and homing. Tibial cortex transverse transport (TTT), a novel surgical technique based on DO principle, has shown promising result in treating peripheral ischemic conditions, including DFU. However, the underlying biological mechanisms for TTT surgery remain unclear. This study aimed to unveil the mechanism by which the TTT technique confers therapeutic benefits for treatment of DFU, with a specific focus on MSCs mobilization and homing.

METHODS: In this study, a novel rat model of TTT was established with a specially designed external fixator to investigate its therapeutic effects on DFU (Figure A). Rats were randomly divided into 3 groups: sham group (negative control), fixator group (surgery without bone cortex transport) and TTT group (surgery with bone cortex transport). Wound healing index (WHI), histology and immunohistochemistry were used to evaluate the wound healing processes. MSCs mobilization and homing were tracked using flow cytometry analysis and immunohistochemistry. Serum was isolated from the peripheral blood of rats in the three groups for in vitro assays. Cell migration experiments were performed to evaluate whether the serum can enhance the migration capacity of rat bone marrow mesenchymal stem cells (BMSCs). qRT-PCR and ELISA assays were conducted to identify specific soluble cytokines in the serum that may enhance BMSCs migration capacity. siRNA was designed to interfere with the expression of selected receptor protein to verify the function of specific gene in BMSCs migration. One-way ANOVAs were performed to compare the result among three groups. A p-value <0.05 was considered as significant. All experiments were approved by the Animal Research Ethics Committee of The Chinese University of Hong Kong (AEEC no. 20-095-ECS).

RESULTS SECTION: TTT technique showed significant benefits in accelerating DFU closure and improving the quality of newly formed skin tissues. Both gross and histological examinations revealed enhanced recovery of the epidermis and dermis, along with increased local collagen deposition following TTT treatment. Additionally, TTT induced the migration of MSCs to the peripheral blood on day 2 after cortex transport, and these mobilized MSCs homed to the site of the chronic wound, thereby accelerating wound healing. Serum isolated from rats treated with TTT significantly promoted mobilization of BMSCs in vitro, which potentially facilitated wound healing. Furthermore, the concentration of stromal cell-derived factor 1 (SDF-1 or CXCL12) protein in the peripheral blood was upregulated by TTT treatment. Importantly, knockdown of CXCR4 receptor abolished the beneficial effects of serum from TTT group on MSCs migration.

DISCUSSION: The TTT technique accelerated DFU healing via enhancing the mobilization and homing of MSCs (**Figure B**). Soluble cytokines, such as SDF-1, appear to play a crucial role in mediating the effect of TTT (**Figure C**). These mobilized MSCs, in turn, may promote angiogenesis and regulate the immune response at the ulcer site potentially through their paracrine factors such as VEGF and IL-10. However, further investigations are required to fully elucidate the specific mechanisms by which mobilized MSCs promote wound healing. Moreover, it is crucial to confirm whether other cytokines in addition to SDF-1 may also contribute to promoting MSCs homing following TTT treatment. Exploring those cytokines would provide a more in-depth understanding of the cytokine-mediated mechanisms underlying MSCs migration and could potentially unveil additional therapeutic targets for DFU management. In conclusion, these findings shed light on the potential biological mechanisms underlying TTT treatment and may pave the way for the development of new therapeutic strategies in DFU management.

SIGNIFICANCE/CLINICAL RELEVANCE: This study offers valuable insights into the underlying biological mechanisms of TTT treatment and have the potential to inform novel therapeutic targets for managing DFU. The findings of this research warrant further exploration to fully leverage the potential of TTT and advance treatment options for patients suffering from DFU.

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IMAGES AND TABLES:

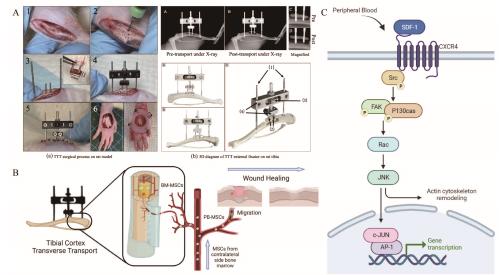


Figure A: Illustration of tibial cortex transverse transport (TTT) external fixator and surgical procedure. (a) TTT surgical process on the rat model: 1) The right tibia was exposed; 2) guided with the external fixator, holes and pinholes were drilled, forming a bone window; 3) four screws were inserted; 4) and 5) the cortical bone chip was dislocated, and the external fixator was assembled and incision was sutured; and 6) a silicone splint was fixed to the wound skin. (b) 3D diagram of TTT external fixator on rat tibia: 1) two long screws; 2) two small screws; 3) a turning nut; and 4) the external fixator frame.

Figure B: TTT technique may promote DFU healing by enhancing MSCs mobilization and homing.

Figure C: SDF-1/CXCR4 axis may enhance MSCs migration capacity via JNK signaling pathway.