

ORIGINAL RESEARCH ARTICLE

Tibial transverse transport promotes diabetic wound healing via angiogenic growth factors, anti-inflammatory response, and progenitor cell mobilization

Shuanji Ou^{1,2†}, **Xiaodong Wu^{1,3†}**, **Jianping Wen¹**, **Jiabao Liu¹**, **Jiatao Lei¹**, **Tingyu He¹**, **Jiafu Xie¹**, **Rongshen Yang¹**, **Wei Zeng¹**, **Changliang Xia^{1,2}**, **Pengcheng Chen^{1,2}**, **Changpeng Xu^{1,2*}**, and **Yong Qi^{1,2*}**

¹Department of Orthopedic, Orthopedic Center, The Affiliated Guangdong Second Provincial General Hospital of Jinan University, Guangzhou, Guangdong, China

²Department of Orthopedic, Guangdong Traditional Medical and Sports Injury Rehabilitation Research Institute, The Affiliated Guangdong Second Provincial General Hospital of Jinan University, Guangzhou, Guangdong, China

³Department of Orthopedic, The First Dongguan Affiliated Hospital of Guangdong Medical University, Dongguan, Guangdong, China

[†]These authors contributed equally to this work.

*Corresponding authors:

Changpeng Xu
(gd2hxc@163.com)
Yong Qi
(yongqi4040@163.com)

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Abstract

Introduction: Tibial transverse transport (TTT), a technique derived from the Ilizarov method, has shown clinical efficacy in treating recalcitrant diabetic foot ulcers by promoting angiogenesis. However, its underlying molecular mechanisms remain unclear.

Objectives: This study aimed to investigate the mechanisms by which TTT influences wound healing in a rat model of type 2 diabetes mellitus (T2DM).

Methods: Thirty-six male Sprague–Dawley rats were induced to develop T2DM via a high-fat diet and low-dose streptozotocin. They were randomized into three groups: sham surgery, external fixator application without transport, and active TTT surgery. A full-thickness excisional wound was created on the dorsum of the right foot. Wound area was measured photographically on days 0, 3, 6, 9, 12, and 15. Serum levels of vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), interleukin-10 (IL-10), and granulocyte colony-stimulating factor (G-CSF) were quantified using enzyme-linked immunosorbent assay on days 9 and 15.

Results: The TTT group exhibited a significantly accelerated reduction in wound area from day 9 onwards compared to the sham and fixator groups ($p < 0.05$). Concomitantly, serum levels of VEGF, bFGF, PDGF, IL-10, and G-CSF were significantly elevated in the TTT group at both time points.

Conclusion: We established a T2DM rat model of TTT surgery. Our findings suggest that the TTT technique accelerates diabetic wound healing, potentially through a multifaceted mechanism involving enhanced serum levels of key angiogenic growth factors, promotion of an anti-inflammatory microenvironment (via IL-10), and mobilization of endothelial progenitor cells (potentially via G-CSF).

Keywords: Tibial transverse transport; Diabetic foot ulcers; Rat model

1. Introduction

Diabetes mellitus (DM) is a global pandemic, affecting approximately 537 million adults worldwide.¹ Diabetic foot ulcers (DFUs), a devastating complication, develop in over 30% of diabetic patients during their lifetime, substantially increasing the risks of amputation and mortality.^{2,3} The pathogenesis of DFUs is multifactorial, involving peripheral neuropathy, macro- and micro-vascular dysfunction, and a persistent pro-inflammatory state. Chronic hyperglycemia leads to endothelial dysfunction, reduced nitric oxide bioavailability, and impaired angiogenesis, critically hindering the wound-healing cascade.^{4,5} The main reason for lower limb amputation in patients with diabetes is the tissue damage caused by peripheral nerve disorders and peripheral vascular diseases. High blood sugar accelerates vascular damage, inhibits collagen synthesis, and affects wound healing.

The condition of patients with diabetic foot is complex, and the treatment is extremely difficult, with effective treatment methods remaining unavailable. Currently, a comprehensive treatment model combining local and systemic treatment and involving multiple disciplines is emphasized. The management of DFUs poses a significant socioeconomic burden, encompassing glycemic control, debridement, advanced dressings, offloading, and revascularization.⁶⁻¹⁰ Despite these interventions, healing recalcitrant DFUs remains a formidable clinical challenge. The lack of improvement in blood circulation to the lower limbs may be the most significant factor affecting treatment outcome. To prevent the progression of the disease, numerous patients have to undergo amputation. The high amputation rate and mortality rate of diabetic foot disease impose a heavy burden on patients and society. The treatment goal for diabetic foot should be limb preservation. However, there is no effective treatment for DFUs, and limb preservation remains a worldwide challenge.

Tibial transverse transport (TTT) is developed based on the bone transport technique founded by Ilizarov.¹¹ Its biological principle is the tension-stress law, which means that biological tissues can stimulate cell division, tissue regeneration, and active growth under continuous, stable, and slow traction. The Ilizarov method, based on the “tension-stress” principle, stimulates tissue regeneration, including bone (distraction osteogenesis) and soft tissues (distraction histogenesis).¹² Before new bone formation in the gap at the osteotomy site during distraction, the microvascular network undergoes active regeneration. Angiography also confirmed the reconstruction of new blood vessels and microcirculation in the distraction area of the limb, improving the blood circulation of the affected

limb. As the bone transport distance increases, the skin and periosteum around the limb will also be stretched. Compared to the contralateral side, the number of blood vessels in the skin tissue per unit area on the transport side of the limb is significantly increased.¹³ The bone transplantation technique utilizes “time variables,” moving the resected bone segments at a rate of 1 mm per day. This chronic pulling force and compressive stress stimulate the regeneration and repair functions of human tissues, enabling them to reach a vigorous state. Most importantly, it establishes a capillary network, and the rich blood supply promotes the transformation, repair, and reconstruction of the tissues. Building on this, TTT was developed, involving the gradual transport of a segment of the tibial cortex. Initially used for thromboangiitis obliterans, TTT demonstrated potent neovascularization around the transport site.¹⁴

Subsequently, TTT has been successfully applied to severe DFUs, with compelling clinical evidence. Recent large-scale studies involving hundreds of patients have reported that TTT significantly improves limb salvage rates, promotes complete wound healing, enhances foot perfusion (measured by transcutaneous oxygen pressure and ankle-brachial index), and improves quality of life.¹⁵⁻²⁰ Our previous clinical research indicated that TTT elevates serum levels of angiogenic factors, such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and platelet-derived growth factor (PDGF).¹⁷ Animal studies, including a canine model and a normal rat model, have confirmed TTT's capacity to induce neovascularization and modulate immune responses.²¹

However, the precise mechanistic pathways by which TTT facilitates healing in the context of diabetic pathophysiology remain poorly elucidated. While a TTT model in normal rats exists,²² its efficacy and mechanism in a diabetic environment, characterized by impaired healing responses, require validation. Given that 90–95% of diabetic cases are type 2,²³ establishing a type 2 DM (T2DM) animal model for TTT is crucial. The combination of a high-fat diet (HFD) and low-dose streptozotocin (STZ) effectively induces a T2DM phenotype in rats, mimicking human disease progression.²⁴ Therefore, this study aims to establish a reproducible T2DM rat model of TTT surgery to explore the molecular mechanisms underlying its therapeutic effects on diabetic wound healing.

2. Materials and methods

2.1. Animals and ethics approval

Male Sprague-Dawley rats ($n = 36$) aged 8 weeks (with body weights ranging from approximately 200–230 g) were obtained from the animal research center of

Southern Medical University. The animals were placed in an environment with a 12-hour alternation between light and darkness each day, and they had free access to food and water. Rats were acclimated for at least 5 weeks before STZ injection. Five rats were housed in each cage, and the environmental conditions were maintained at $21 \pm 1^\circ\text{C}$ and $55 \pm 5\%$ humidity, with a 12-hour light–dark cycle (lights on 08:00–20:00). All experimental procedures were approved by the Animal Ethics Committee of The Second People's Hospital of Guangdong Province (Approval No. 2022-DW-KZ-006-01) and conducted in accordance with relevant guidelines.

2.2. Induction of type 2 diabetes mellitus

Rats were fed a 45% HFD (recipe ingredients: 2.5% cholesterol, 10% lard, 1% bile salt, 20% sucrose, and 66.5% regular feed) for 5 weeks to induce insulin resistance. Following an overnight fast, they received a single tail vein injection of freshly prepared STZ (35 mg/kg in 0.1 M citrate buffer, pH 4.5). One week post-injection, rats with fasting blood glucose levels persistently > 16.7 mmol/L were considered diabetic and included in the study.²⁵

2.3. Experimental design and surgical groups

Diabetic rats were allowed to recover for at least 1 week before the experiment. All experimental rats were anesthetized and operated in a sterile operating room to minimize postoperative complications. The diabetic rats were randomly divided into three groups (with 12 rats in each group):

- Sham group: Underwent a skin incision and wound creation only.
- Fixator group: Underwent application of the external fixator device and wound creation, but without corticotomy or bone transport.
- TTT group: Underwent the complete TTT surgical procedure and wound creation.

2.4. Tibial transverse transport surgery

The TTT operation was conducted in accordance with the method described in a previous study.²⁶ A modified miniaturized external fixator (China Patent 202220302485.X) was used (Figure 1A). Under isoflurane anesthesia, a 2.5 cm curved incision was made over the proximal right tibia. During the operation, cortical osteotomy was performed on the middle and upper segments of the tibia. Guided by the fixator, three screw entry points and a rectangular corticotomy window (5.0 mm height $\times$ 4.0 mm width) on the anteromedial cortex were marked. Holes were drilled, and the corticotomy was completed with a scalpel, preserving the medial border.

The external frame was percutaneously fixed with three screws (1 mm diameter). Finally, the medial border was osteotomized to complete the transport segment (Figure 1B).

2.5. Dorsal foot wound model

Immediately after TTT or control surgeries, a full-thickness excisional wound (4.0 $\times$ 5.0 mm) was created on the shaved and disinfected dorsum of the right hind paw using a sterile template (Figure 1B).

2.6. Bone transport protocol

After a 3-day latency period, active transverse transport of the bone segment commenced at a rate of 0.25 mm every 12 hours (0.5 mm/day) for 3 days laterally, then stop for 3 days, followed by 3 days of medial transport (return), totaling a 9-day active transport period. Transport was confirmed radiographically (Figure 1C). No obvious infections or other complications were observed in all experimental animals after surgery. For each group of diabetic rats, they were euthanized using a carbon dioxide euthanasia device (Biowill, BM Shanghai Biowill Co., Ltd., China) at a 6.5 L/min flow rate on the 9th and 15th days after the surgery. Skin damage photos were taken, and serological examinations were conducted.

2.7. Wound healing assessment

Digital photographs of wounds were taken on days 0, 3, 6, 9, 12, and 15 postoperatively. The wound area was measured using ImageJ software (Version 1.53, National Institutes of Health, United States), and the percentage of wound closure compared to the 0th day was calculated.

2.8. Enzyme-linked immunosorbent assay

Blood samples were collected from the tail vein on days 9 and 15. Serum was separated and stored at -80°C . Concentrations of VEGF, bFGF, PDGF, interleukin (IL)-10, and granulocyte colony-stimulating factor (G-CSF) were determined using specific commercial enzyme-linked immunosorbent assay kits (CEK1755, Colorful-Gene Biotech, China) according to the manufacturer's protocols.

2.9. Statistical analysis

Data are presented as mean $\pm$ standard error of the mean. Differences in blood glucose before and after STZ injection were analyzed using a paired *t*-test. To compare multiple groups at a specific time point, a one-way analysis of variance was employed, followed by Tukey's post-hoc test. A *p*-value < 0.05 was considered statistically significant. All analyses were conducted using Statistical Package for Social Sciences (SPSS 25.0, IBM Corp., USA).

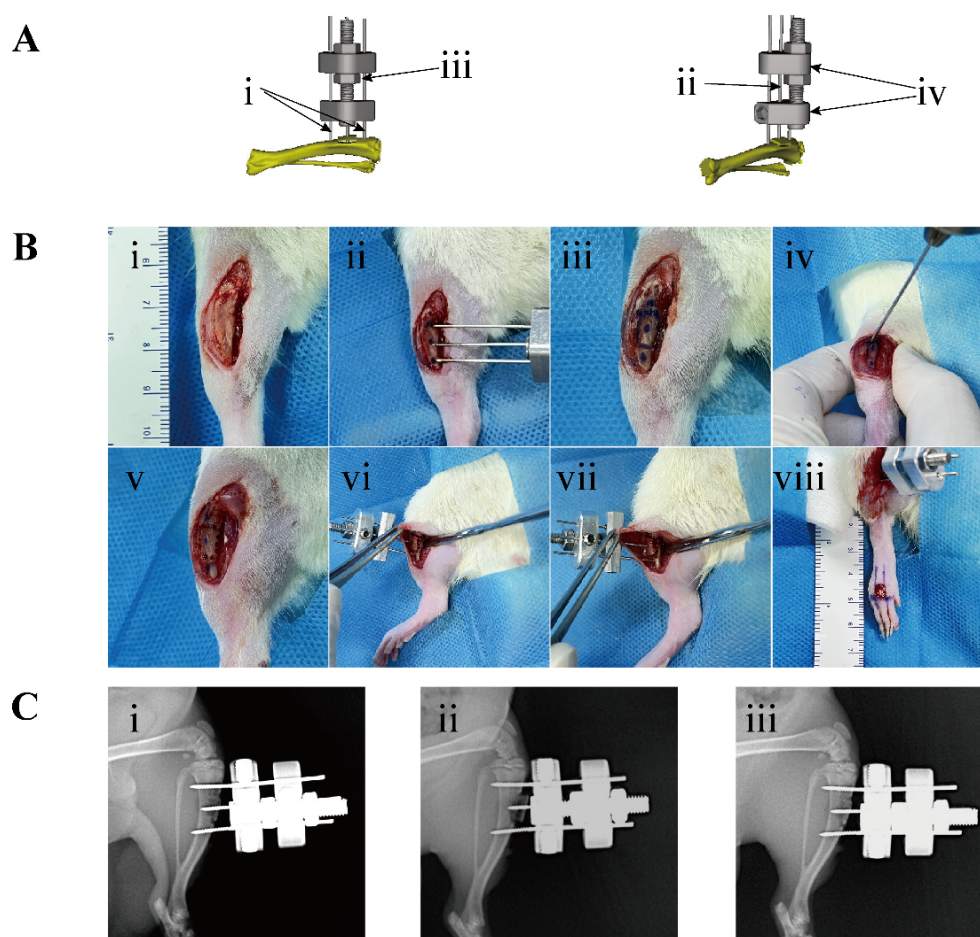


Figure 1. Introduction of the modified external fixator device for rats, surgical procedure, and X-ray. (A) Display of external fixator: (i) two screws for fixing the external frame; (ii) one screw for bone transport; (iii) two nuts for moving cortical bone; (iv) triangular external fixator frame. (B) Tibial transverse transport surgical procedure and foot wound model: (i) A 2.5-cm curved skin incision was made over the proximal tibia; (ii), (iii) guided with the external fixator, three entry points and a rectangular corticotomy window were marked; (iv), (v) holes along the scribed lines were drilled; (vi) rectangular corticotomy window was created and the triangular external fixator frame was fixated; (vii) cortex transverse transport was confirmed by turning the nuts; (viii) a full-thickness excisional wound (4.0 × 5.0 mm) created on the dorsum. (C) X-rays were performed on days (i) 3, (ii) 6, and (iii) 12.

3. Results

3.1. Characterization of the type 2 diabetes mellitus model

Following STZ injection, rats developed stable hyperglycemia (Table 1) and exhibited classic diabetic symptoms, including polydipsia, polyphagia, polyuria, and reduced weight gain. No major surgical complications (e.g., pin-site infection, fracture, or loose external fixed frame) were observed.

3.2. Tibial transverse transport accelerates wound closure in diabetic rats

The healing process of the rat wound went through the following stages: Firstly, there was an inflammatory stage,

during which the dermis of the wound contracted, the area decreased, the surface was covered by necrotic tissue, and a scab formed; then it entered the proliferation stage, where the epidermis at the wound edge gradually proliferated and migrated, the necrotic tissue decreased, and the wound gradually shrank; finally, it was the reconstruction stage, during which the wound significantly shrank and the healing was gradually completed. Based on the wound healing process, we selected five time points, namely 3, 6, 9, 12, and 15 days after the wound was introduced, for observation and evaluation. On the 9th, 12th, and 15th days, the percentage of wound area in the TTT group was significantly lower than that in the fixator group and the sham group. On the 9th day, the wound area was lower in the TTT group than in the fixator group ($p = 0.038$)

Table 1. Changes in blood glucose levels

Group	Before induction	One week after STZ injection	9 days after the operation	15 days after the operation
Sham	4.38 ± 0.18	30.48 ± 1.04	30.12 ± 0.95	29.48 ± 1.21
Fixator	4.32 ± 0.27	29.67 ± 1.03	26.89 ± 1.63	28.10 ± 1.81
TTT	4.47 ± 0.24	28.45 ± 1.29	26.52 ± 1.41	27.39 ± 1.38

Notes: Blood glucose levels (mmol/L) increased significantly 1 week after STZ injection, and sustained hyperglycemia after the operation; Values are shown as mean ± standard error; $n = 12$; all blood glucose levels at days 9 and 15 after the operation showed no significant difference compared to one week after STZ injection ($p > 0.05$).

Abbreviation: STZ: Streptozotocin.

and the sham group ($p = 0.009$). Similar differences were observed on the 12th day (vs. fixator, $p = 0.040$; vs. sham, $p = 0.003$) and day 15 (vs. fixator, $p = 0.043$; vs. sham, $p = 0.004$) (Figure 2B). The above results indicate that TTT plays a significant role in promoting the repair of diabetic refractory wounds and demonstrates a strong effect even at the early stage of healing.

3.3. Tibial transverse transport elevates serum levels of healing-related cytokines

Enzyme-linked immunosorbent assay detected a significant increase in serum levels of key cytokines in the TTT group compared to the sham and fixator groups on both day 9 and day 15 (Figure 3). Specifically, serum levels of the angiogenic factors (VEGF, bFGF, and PDGF), the anti-inflammatory cytokine (IL-10), and the hematopoietic factor (G-CSF) were all markedly increased following TTT surgery. The above results indicate that TTT promotes the release of various growth factors that facilitate wound healing. These factors, by stimulating angiogenesis, cell proliferation, and collagen deposition, may provide an important biological basis for wound repair.

4. Discussion

Wound healing refers to a series of pathological and physiological processes in which local tissues regenerate, repair, and reconstruct in response to injury factors. The inflammatory response phase, the cell proliferation phase, and the tissue remodeling phase are three independent and overlapping stages of wound healing, which is a precise integration of biological and molecular events. Once tissue damage occurs, the first to react are platelets, which form fibrin blood clots to stop bleeding at the wound site. Platelets also release various cytokines, mediating the migration of macrophages and fibroblasts to the wound. The inflammatory response begins with the aggregation of neutrophils, macrophages, and lymphocytes at the wound site. The cell proliferation phase largely overlaps with the inflammatory response phase, including cell

migration, capillary formation, collagen deposition, and extracellular matrix resynthesis. Fibroblasts and other tissue cells produce new growth factors, and epithelial cells begin to migrate and form new blood vessels. Fibroblasts, keratinocytes, and new blood vessels migrate to the wound site to produce granulation tissue. After the proliferative phase, collagen begins to remodel, accompanied by the maturation and regression of blood vessels. Changes in internal and external environments may cause physiological controllable factors to evolve into pathological uncontrollable factors, leading to delayed or non-healing of wounds.

Delayed wound healing and even long-term non-healing of DFUs are common clinical phenomena and also the focus and difficulty in wound treatment. Microvascular disease and peripheral neuropathy are the underlying pathologies of diabetic foot. Disordered inflammatory response, reduced granulation tissue, impaired wound vascularization, and deficiencies or dysfunctions of growth factors are the main pathological processes in diabetic foot. Wound cells, vascular factors, cytokines, and the extracellular matrix are high-risk factors for the difficult healing of diabetic foot. Currently, it is believed that prolonged non-healing of DFUs may be related to the accumulation of advanced glycation end products (AGEs), dysfunction of vascular endothelial cells, dysregulation of NO in blood vessels, imbalance of inflammatory factor response, and abnormal expression of cytokines and their receptors. Under the condition of persistent hyperglycemia, the aldehyde group of sugar combines with the amino group of protein to produce AGEs. The skin tissue is rich in collagen and is a good site for glycation. The accumulation of AGEs in skin tissue leads to changes in tissue cellular behavior, which has attracted widespread attention. AGEs are mainly covalent products formed by the reaction of glucose and the amino group of protein through non-enzymatic reactions, forming a Schiff base first, followed by Amadori rearrangement to form a relatively stable aldehyde-amine product, and then

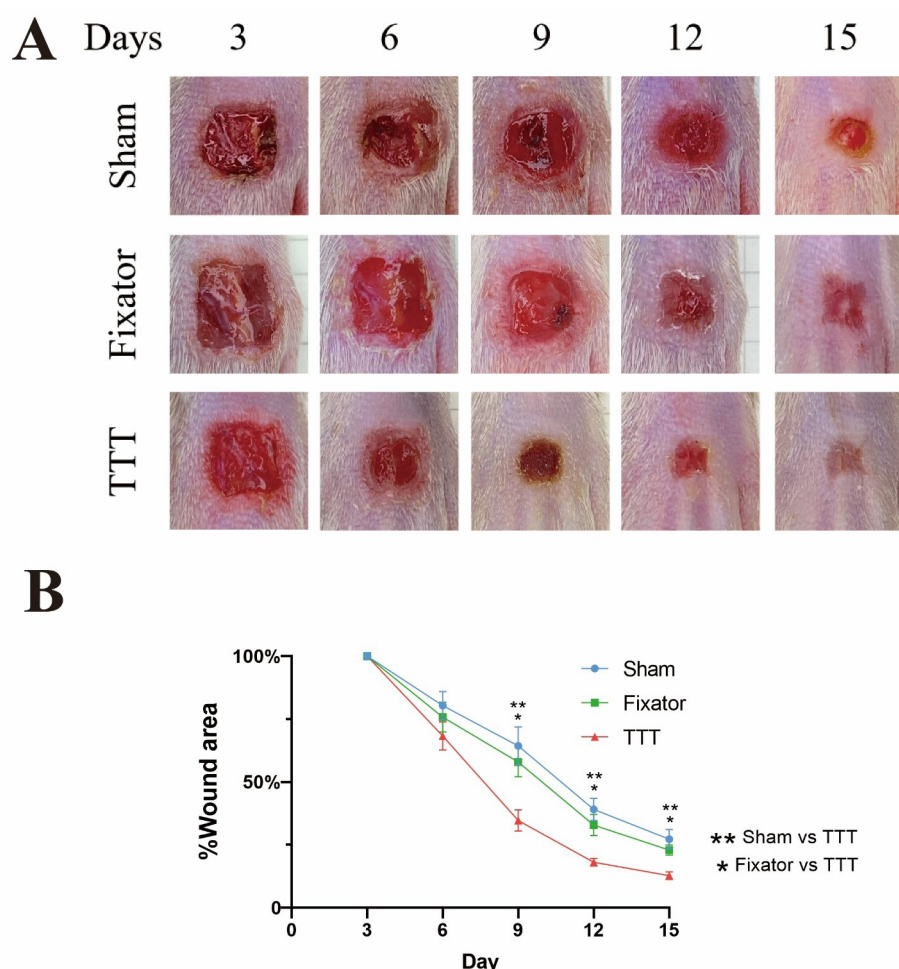


Figure 2. Tibial transverse transport (TTT) improved the healing of wounds on the dorsal foot of type 2 diabetic rats. (A) Representative photographs of diabetic wound healing in the sham group, fixator group, and TTT group show a significant difference beginning at day 9. (B) Changes in wound area of the sham group, fixator group, and TTT group on days 3, 9, 12, and 15.

Notes: Values are shown as mean $\pm$ standard error, $n = 12$; * $p < 0.05$; ** $p < 0.01$.

intermolecular dehydration to form AGEs, which is called the Maillard reaction.²⁶

The vascular regeneration technology developed based on the chronic stretching tissue regeneration biology principle is a technique that uses the lateral bone transplantation surgical method to repair and reconstruct limb functions through vascular regeneration, and is used to treat lower limb ischemic diseases. Traction-based tissue regeneration technology involves applying appropriate tensile stress to stimulate bone and soft tissue regeneration. In recent years, there have been significant advancements in the medical concepts, technological innovations, and clinical applications of traction osteogenesis technology, which has even saved some cases of lower limb vascular diseases that were on the verge of amputation. A series of fundamental research and clinical practices has proven that

when living tissues are subjected to appropriate tractional stress, they can activate gene expression and stimulate tissue regeneration.²⁷ TTT is based on the law of tensile stress principle. Under the stimulation of appropriate tensile stress, limb tissues have the ability to regenerate. During traction osteogenesis, extensive new blood vessels form, and this surgical method is used to treat DFUs.^{28,29}

Normal wound healing progresses through coordinated phases of hemostasis, inflammation, proliferation, and remodeling. Diabetes disrupts this sequence through hyperglycemia-induced neuropathy, microangiopathy, and immune dysfunction. The HFD/STZ-induced T2DM rat model recapitulates these impairments, providing a valid platform for therapeutic investigation. The observed delay in wound healing in the sham and fixator groups contrasted sharply with the improvement seen in the

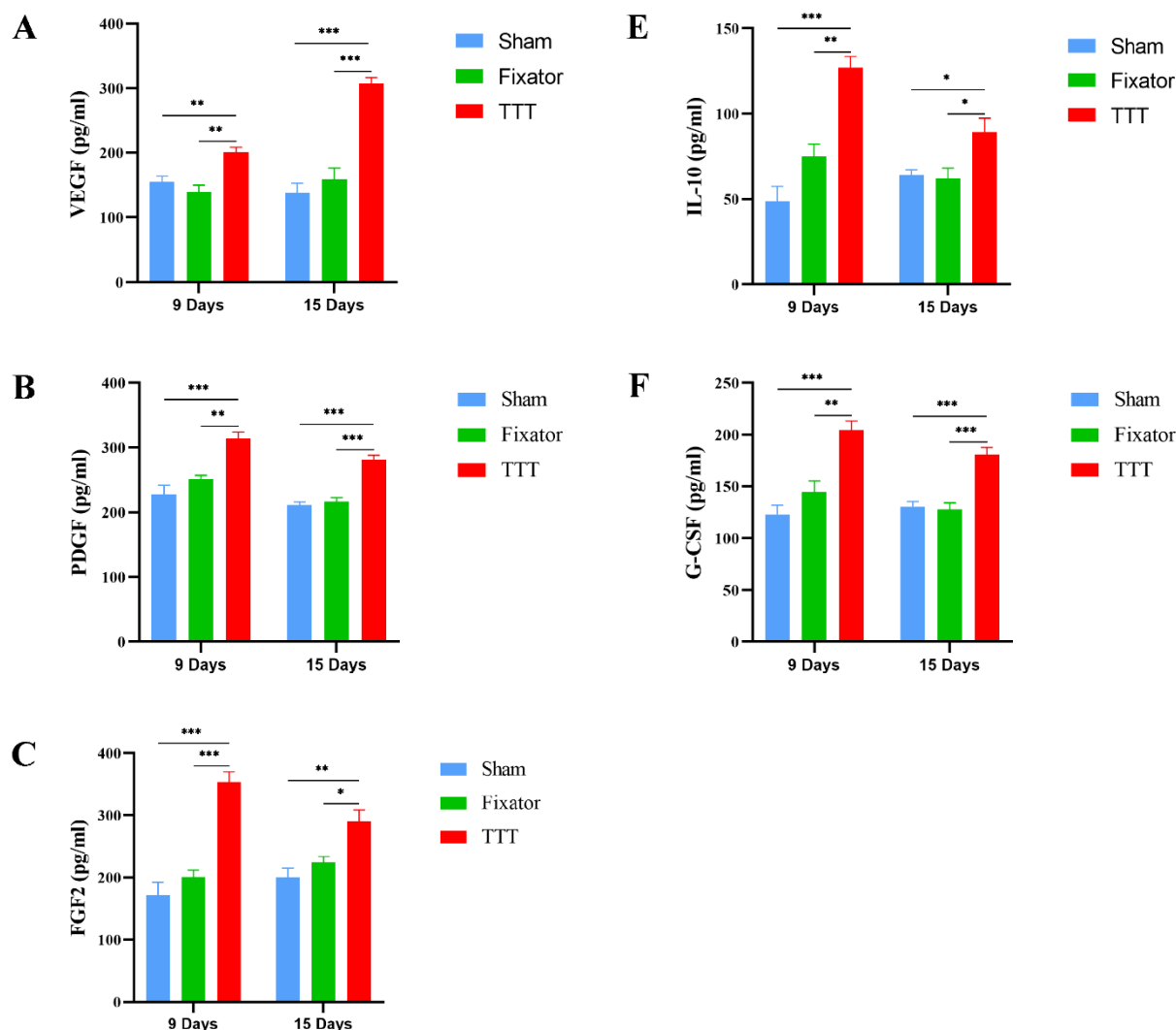


Figure 3. TTT increased cytokine levels in type 2 diabetic rats during wound healing. Notes: Values are shown as mean $\pm$ standard error; $n = 12$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Abbreviations: bFGF: Basic fibroblast growth factor; G-CSF: Granulocyte colony-stimulating factor; IL-10: Interleukin-10; PDGF: Platelet-derived growth factor; TTT: Tibial transverse transport; VEGF: Vascular endothelial growth factor.

TTT group from day 9, aligning with the timeline of active mechanical stimulation and previous reports. It is possible that repeated mechanical traction induced by the transverse bone repositioning stimulates vascular regeneration, improves blood circulation in the lower leg and foot, and thereby promotes the healing of ulcerated wounds in diabetic rats. We observed that during transverse bone repositioning, the tissue at the foot wound gradually turned bright red, became soft and moist, the skin around the wound gradually moved closer to the wound, and the wound was successfully repaired.

Tibial transverse transport can effectively treat

diabetic foot, but the mechanism by which it stimulates angiogenesis and promotes wound healing remains unclear. Wound healing requires the participation of various tissue cells, extracellular matrix, and cytokines, and is a highly complex biological process. Angiogenesis is a crucial factor in wound healing and is closely related to angiogenesis-related factors. These factors (VEGF, bFGF, and PDGF) can not only stimulate blood vessel formation but also promote cell proliferation and the synthesis of extracellular matrix proteins, playing an important role in wound repair. Angiogenesis-related factors are important substances for wound healing, and the difficulty in healing DFUs may be related to insufficient secretion of these

factors.

Vascular endothelial growth factor is a highly specific mitogen that acts as a cytokine to stimulate the growth of vascular endothelial cells. It promotes the proliferation and migration of vascular endothelial cells, enhances vascular permeability, regulates wound vascularization, and plays a crucial role in the induction of new blood vessel formation. It maintains the normal state and integrity of blood vessels. VEGF binds to VEGF receptors on the endothelial cell membrane, inducing receptor phosphorylation, activating mitogen-activated protein kinases, and conferring VEGF's mitogenic properties. This induces endothelial cell proliferation. VEGF can also enhance the activity of plasminogen activator and plasminogen inhibitor-1 by increasing their mRNA expression, thereby promoting the hydrolysis of extracellular proteins and facilitating the formation of new blood vessels.³⁰ VEGF plays an important role in restoring and maintaining the integrity of vascular endothelial cells. It protects endothelial cells from programmed cell death, stimulates nitric oxide production, dilates vascular smooth muscle cells, promotes blood flow recovery, and increases the blood flow in collateral circulation. Diabetic patients have vascular endothelial dysfunction. The occurrence and progression of diabetic foot are closely related to decreased VEGF levels, leading to impaired wound healing. VEGF can induce the formation of blood and lymphatic vessels at diabetic wound sites, and attract inflammatory cells, thereby accelerating the healing of diabetic wounds.³¹ VEGF is an indispensable cytokine in the wound healing process and participates in all stages of wound repair. Local application of VEGF can vascularize the wound site and enhance wound healing.

The significant elevation of VEGF in the TTT group aligns with our clinical observations and underscores TTT's pro-angiogenic role. Mechanical stimulation during bone repositioning can maintain relatively high serum VEGF levels. Repeated tensile stress stimulation does not cause a continuous increase in VEGF, but instead keeps VEGF at a relatively constant level. Performing bone repositioning in an accordion-like manner requires a longer period of continuous and slow mechanical stimulation. This stimulation can promote and maintain VEGF levels. During bone repositioning, VEGF is consistently highly expressed, promoting angiogenesis, reconstruction, and the restoration of blood flow. New blood vessels can provide nutrients for repairing damaged tissues and help promote the healing of diabetic foot wounds, thereby achieving the goal of treating DFUs. We observed vascular regeneration at the ulcer surface of DFUs. The skin around the ulcer migrated and repaired towards the central area. During the ulcer healing process, the ulcer surface gradually

developed from pale ischemia to a more bleeding-prone state. This may be related to VEGF levels being stimulated by bone repositioning.

Basic fibroblast growth factor is a member of the fibroblast growth factor family. It is a heparin-binding protein distributed in normal tissues and promotes cell division. It is an important factor in promoting wound healing. bFGF can induce the proliferation and differentiation of various cells and has the functions of stimulating endothelial cell division, promoting the formation of new blood vessels, promoting wound healing and tissue repair, promoting embryonic tissue development and differentiation, and participating in nerve regeneration. bFGF plays an important role in wound repair by promoting fibroblast proliferation, collagen synthesis, and endothelial cell vascularization.³² As a mitogen, it can promote the proliferation of various cell types, including epidermal, mesenchymal, and endothelial cells, by regulating protein signaling. Wound healing can be promoted by appropriately regulating bFGF release and stimulating the infiltration of capillary endothelial cells, thereby increasing the vascularization and thickness of granulation tissue.³³ bFGF is an important cytokine in the wound-healing process, regulating the growth factors and extracellular matrix necessary for wound healing. As a chemotactic factor for endothelial cell proliferation, it can directly promote endothelial cell proliferation and regulate endothelial cell VEGF secretion, thereby inducing the formation of new blood vessels.³⁴

The experimental results of this study show that the serum bFGF levels in the TTT group were statistically significantly higher compared to other groups ($p < 0.05$), suggesting that the transverse bone transplantation of the tibia can promote the expression of bFGF by repeatedly moving the tibial osteotomy blocks. The transverse bone transplantation of the tibia can also maintain a relatively high level of bFGF. Repeated mechanical stimulation during bone transplantation can maintain high serum bFGF levels, and the Ilizarov method, which provides a longer period of continuous, slow mechanical stimulation, can promote and maintain bFGF levels and accelerate the healing of DFUs.

Platelet-derived growth factor is mainly produced by fibroblasts and platelets. It is an alkaline polypeptide growth factor that can enhance the chemotactic responsiveness of cells, stimulate cell proliferation, accelerate the formation of granulation tissue, and promote wound healing. PDGF can promote the infiltration of neutrophils and macrophages into the damaged area to exert debridement effects, and promote the secretion of new extracellular matrix and insulin-like growth factor-1 via fibroblasts to

mediate re-epithelialization. PDGF can only exert its effect by binding to the specific PDGF receptor on the target cell membrane, initiating and rapidly repairing the injured tissue.³⁵ PDGF can regulate macrophage function. In a diabetic environment, this regulation may fail, preventing macrophages from effectively transitioning from an inflammatory (M1) to a reparative (M2) phenotype, thus keeping the wound in an inflammatory state. PDGF plays a key role in all stages of wound healing and is an essential growth factor for early wound healing. It plays a significant role in promoting macrophage-mediated tissue debridement and granulation tissue formation, thereby supporting wound healing, and also serves as an auxiliary factor in stabilizing blood vessels. It plays an important role in angiogenesis and the growth of existing vascular tissues. The PDGF produced by microvascular cells can induce VEGF, suggesting that PDGF plays an important role in angiogenesis.³⁶

This study focused on diabetic rats. The serum levels of PDGF in the TTT group were higher than those in the control group ($p < 0.05$), suggesting that the transverse bone repositioning surgery through the repeated repositioning of the tibial osteotomy blocks can increase serum PDGF levels. This indicates that this treatment method can maintain PDGF levels, thereby facilitating DFU healing. TTT-induced mechanical stress is likely a potent stimulus for the sustained release of these factors from various cells, including osteocytes, fibroblasts, and macrophages. During angiogenesis and wound healing, angiogenic-related factors interact and promote each other. VEGF can promote the formation of new blood vessels, but it does not play a role in the mature process of re-epithelialization. PDGF can promote the epithelialization of wound granulation tissue and does not play a dominant role in inducing new blood vessel formation. bFGF can also increase VEGF levels, thereby inducing angiogenesis.

Interleukin-10, as a crucial anti-inflammatory factor, plays a central role in regulating immune responses and promoting tissue repair. In the development of DFUs, insufficient IL-10 production, impaired signal transduction, and IL-10-mediated imbalance of immune homeostasis are important mechanisms that drive chronic inflammation in the wound and make healing difficult. Local IL-10 production in the wound is relatively deficient. The high-glucose environment of diabetes and the persistent damage-related molecular patterns drive macrophages to polarize towards the pro-inflammatory M1 phenotype, while blocking the transformation to the anti-inflammatory and repair M2 phenotype, resulting in reduced secretion of repair-related cytokines such as IL-10.³⁷ Additionally, the impaired function of regulatory T cells further

weakens the important source of IL-10. Additionally, the IL-10-mediated anti-inflammatory and repair signaling pathways are impaired. IL-10 exerts its biological effects by activating the Janus kinase 1/signal transducer and activator of transcription 3 (STAT3) signaling pathway. Under diabetic conditions, high glucose and oxidative stress can inhibit the phosphorylation and nuclear translocation of STAT3, leading to failure of downstream target gene transcription (such as genes promoting cell migration and inhibiting inflammation), resulting in IL-10 resistance.³⁸ Furthermore, an imbalance in IL-10 function leads to deterioration of the immune microenvironment. The lack of IL-10 reduces its inhibitory effect on the production of pro-inflammatory factors, such as tumor necrosis factor- α , IL-1 β , and IL-6, allowing neutrophils and other inflammatory cells to continuously and over-release proteases (such as matrix metalloproteinases), degrading extracellular matrix and growth factors. Moreover, IL-10 deficiency also impairs macrophage clearance of apoptotic cells (efferocytosis), leading to secondary necrosis and exacerbating inflammation.

The serum IL-10 level in the TTT group was elevated, suggesting that transverse bone repositioning surgery of the tibia can promote IL-10 expression by repeatedly repositioning the tibial osteotomy blocks. Mechanical stimulation can maintain relatively high serum IL-10 levels, and the repositioning of bone in an accordion-like manner requires a longer period of continuous and slow mechanical stimulation. This stimulation can promote and maintain serum IL-10 levels. During the bone repositioning process, IL-10 remains highly expressed, promoting angiogenesis, reconstruction, and restoring blood flow. The new blood vessels can provide nutrients for repairing damaged tissues and promote wound healing.

The marked increase in the anti-inflammatory cytokine IL-10 in the TTT group suggests an immunomodulatory mechanism. Macrophages, pivotal in healing, can polarize towards a pro-healing M2 phenotype, characterized by high IL-10 and VEGF secretion, which resolves inflammation and promotes tissue repair.³⁹ Mechanical stimulation has been shown to direct macrophage polarization towards the M2 phenotype.⁴⁰ Thus, TTT may facilitate the transition from the inflammatory to the proliferative phase by fostering an anti-inflammatory microenvironment.

Granulocyte colony-stimulating factor is a crucial hematopoietic growth factor that primarily stimulates the production and release of neutrophils by the bone marrow and enhances their chemotaxis, phagocytosis, and bactericidal activities. In the development of diabetic foot, G-CSF plays a complex, dual role, closely related to the specific metabolic disorders and chronic inflammatory

environment characteristic of diabetes. G-CSF may have potential protective effects. Diabetic foot patients often have neutrophil functional defects and relatively insufficient numbers, resulting in a decreased ability to fight infections. G-CSF can help control foot infections by increasing circulating neutrophil levels and enhancing their function, thereby creating favorable conditions for ulcer healing. In the pathological background of persistent high blood sugar and chronic low-grade inflammation in diabetes, the effect of G-CSF may be distorted or have adverse effects. Excessive or persistent G-CSF signals may exacerbate neutrophil-mediated tissue inflammation. Activated neutrophils release large amounts of reactive oxygen species and proteases, disrupting tissue repair, aggravating microvascular endothelial damage, and possibly delaying the healing of chronic wounds. This pro-inflammatory effect is particularly prominent in chronic DFUs that are difficult to heal. G-CSF, as an important factor in enhancing the host's defense against infections, may also exacerbate chronic inflammation and tissue damage in the specific microenvironment of diabetes.

Furthermore, the significant rise in G-CSF is particularly intriguing. G-CSF is a potent mobilizer of bone marrow-derived endothelial progenitor cells (EPCs) via the stromal cell-derived factor-1 α /C-X-C chemokine receptor type 4 axis.⁴¹ EPCs are crucial for vascular repair, and their number and function are diminished in diabetes.⁴² While G-CSF therapy has shown promise in DFU treatment, its endogenous upregulation by a mechanical intervention like TTT represents a novel finding. Coupled with elevated VEGF, which also promotes EPC mobilization via the VEGF/endothelial nitric oxide synthase pathway, our data suggest that TTT may systemically enhance the recruitment of reparative cells to the wound site. The serum G-CSF level in the TTT group was elevated, suggesting that the transverse bone repositioning surgery of the tibia can promote the expression of G-CSF by repeatedly moving the tibial osteotomy blocks. Mechanical stimulation can maintain relatively high serum G-CSF levels. This stimulation can promote and maintain serum G-CSF levels, thereby exerting effects on angiogenesis and immune regulation and facilitating wound healing.

This study proposes a hypothetical mechanism by which TTT therapy promotes tissue repair and regeneration, as illustrated in Figure 4. This mechanism highlights the cascading and synergistic interactions among inflammatory modulation, angiogenesis, and hematopoietic/progenitor cell mobilization that collectively drive the tissue microenvironment toward repair and regeneration.

At the initial stage of therapy, TTT therapy is likely to play a critical role by regulating inflammatory responses.

A moderate inflammatory response is considered essential for initiating tissue repair, whereas excessive or prolonged inflammation can delay healing. The proposed mechanism suggests that TTT therapy fine-tunes the regulation of early inflammation by inducing IL-10 expression. As a key immunoregulatory cytokine, IL-10 suppresses the release of pro-inflammatory mediators, limits excessive immune cell activation, and creates a favorable microenvironment for subsequent repair processes. Beyond its role in inflammatory regulation, IL-10 may further promote the activation of angiogenesis-related pathways.

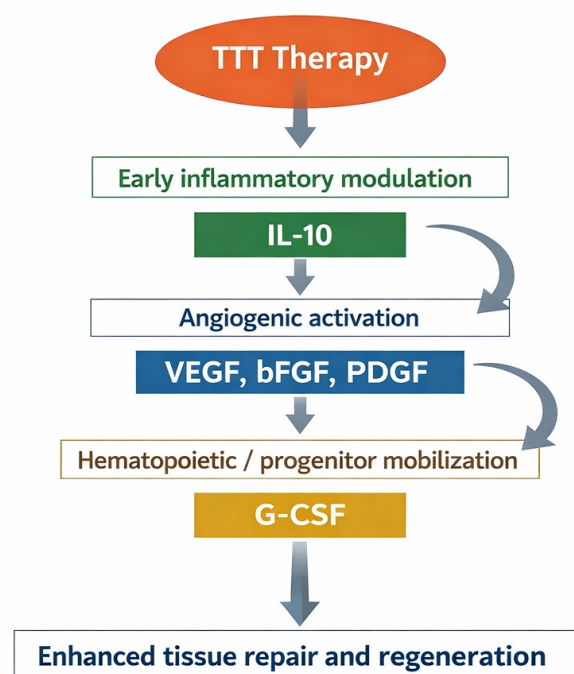


Figure 4. Hypothetical mechanism underlying TTT therapy-mediated tissue repair and regeneration. TTT therapy is proposed to initiate early inflammatory modulation characterized by increased IL-10 expression, leading to controlled immune responses. Subsequent angiogenic activation involves upregulation of key growth factors, including VEGF, bFGF, and PDGF, which support neovascularization and tissue microenvironment remodeling. These angiogenic signals, together with G-CSF-mediated hematopoietic and progenitor cell mobilization, collectively contribute to enhanced tissue repair and regeneration.

Abbreviations: bFGF: Basic fibroblast growth factor; G-CSF: Granulocyte colony-stimulating factor; IL-10: Interleukin-10; PDGF: Platelet-derived growth factor; TTT: Tibial transverse transport; VEGF: Vascular endothelial growth factor.

Angiogenesis is an indispensable component of tissue repair and regeneration, as it supports cell proliferation and functional recovery by improving local blood supply and oxygenation. In the proposed mechanism, angiogenic activation is primarily achieved by upregulating key growth factors, including VEGF, bFGF, and PDGF. These

factors not only promote neovascularization but also enhance vascular stability and regulate the migration and proliferation of fibroblasts and endothelial cells. Notably, enhanced angiogenic signaling may be closely associated with the mobilization of hematopoietic and progenitor cells. VEGF, bFGF, and PDGF have been reported to modulate the bone marrow microenvironment, thereby facilitating the mobilization of hematopoietic stem/progenitor cells into the peripheral circulation. In this hypothesis, G-CSF is considered a key mediator.

This study has several limitations. First, while the excisional wound model in diabetic rats is widely used, it does not fully replicate the complexity of chronic, ischemic DFUs. Second, our findings are correlative; further *in vivo* and *in vitro* studies using specific inhibitors or knockout models are needed to establish causal relationships within the proposed mechanistic network. Third, we did not assess the effect of TTT on diabetic neuropathy, a key component of DFU pathogenesis, which has been reported to improve clinically after TTT. Future studies should include functional and histological evaluations of nerve regeneration.

5. Conclusion

In conclusion, we developed a reproducible T2DM rat model of TTT surgery. Our results demonstrate that TTT effectively promotes diabetic wound healing and systemically upregulates a spectrum of cytokines involved in angiogenesis (VEGF, bFGF, and PDGF), anti-inflammation (IL-10), and progenitor cell mobilization (G-CSF). These findings provide valuable mechanistic insights into the clinical efficacy of TTT for treating recalcitrant DFUs and highlight its potential as a multi-modal therapy targeting several key pathological deficits in diabetic wound healing.

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Conflict of interest

The authors declare that there is no duality of interest associated with this manuscript.

Author contributions

Conceptualization: Jianping Wen, Jiabao Liu

Formal analysis: Wei Zeng, Changliang Xia, Pengcheng Chen

Investigation: Jiatao Lei, Tingyu He

Methodology: Jiafu Xie, Rongshen Yang

Writing—original draft: Shuanji Ou, Xiaodong Wu

Writing—review & editing: Changpeng Xu, Yong Qi

Ethics approval and consent to participate

This study was approved by the Guangdong Second Provincial General Hospital Experimental Animal Ethics Committee (2022-DW-KZ-006-01).

Consent for publication

Not applicable.

Availability of data

No datasets were generated or analyzed during the current study.

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