Far-Infrared Therapy Accelerates Diabetic Wound Healing via Recruitment of Tissue Angiogenesis in a Full-Thickness Wound Healing Model in Rats

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Abstract: Far-infrared ray (FIR) therapy has been applied in the tissue regeneration field. Studies have revealed that FIR could enhance wound healing. However, the biological effects of FIR on diabetic wounds remain unclear. Our study aims to investigate whether FIR could accelerate diabetic wound healing and analyze the biomechanisms. A dorsal skin defect (area, $6 \times 5$ cm$^2$) in a streptozotocin (STZ)-induced diabetes rodent model was designed. Thirty-two male Wistar rats were divided into 4 groups (n = 8 each subgroup). Group 1 consisted of sham, non-diabetic control; group 2, diabetic control without treatment; group 3, diabetic rats received 20 min FIR (FIR-20, 20 min per session, triplicate/weekly for 4 weeks) and group 4, diabetic rats received 40 min FIR (FIR-40, 40 min per session, triplicate in one week for 4 weeks). The wound healing was assessed clinically. Skin blood flow was measured by laser Doppler. The vascular endothelial growth factor (VEGF), 8-hydroxy-2-deoxyguanosine (8-OHdG), eNOS, and Ki-67, were analyzed with immunohistochemical (IHC) staining. Laser Doppler flowmetry analysis of the blood flow of wounding area revealed the blood flow was higher in diabetic rats who received 40 min FIR (FIR-40) as compared to that in FIR-20 group. The wounding area was significantly reduced in the FIR-40 group than in the diabetic control groups. Treatment with the optimal dosage of FIR showed significant increases in angiogenesis expressions (VEGF, eNOS, and EGF), cell proliferation (Ki-67), and suppressed inflammatory response and oxygen radicles (CD45, 8-OHdG) expressions in the FIR-treated groups as compared to that in controls. Treatment with the optimal dosage of FIR significantly facilitated diabetic wound healing and associated with suppressed pro-inflammatory response and increased neovascularization and tissue regeneration.

Keywords: far infrared; diabetic wound healing; angiogenesis

1. Introduction

Chronic wounds occur commonly and reduce the quality of life of those affected, posing a relevant clinical and socioeconomic burden. The healing of a skin wound is a complex multistep process that involves the integration of activities of a variety of tissue and cell types [1]. Diabetic foot ulcers are a major complication of diabetes mellitus and
probably the major component of the diabetic foot [2,3]. Re-epithelialization, a key process in the early stage of wound healing, is the result of the migration and proliferation of keratinocytes in the epidermal layer of the skin around the wound. Many key signaling pathways are activated during wound healing; these pathways include the Wnt/β-catenin, mitogen-activated protein kinase-related pathway, and vascular endothelial growth factor (VEGF) pathways [1,4,5].

Photobiomodulation (PBM) therapy is a form of light therapy that uses non-ionizing forms of light sources in the visible and infrared spectrum, including lasers, LEDs, and broadband light [6,7]. PBM can bring beneficial therapeutic effects, including relief of pain or inflammation, immunomodulation, and promotion of tissue regeneration and wound healing [1,8]. Recently, much attention has been paid to that far-infrared radiation (FIR) may promote wound healing by stimulating the secretion of transforming growth factor β1 (TGF-β1) or activating fibroblasts [9]. However, FIR still knows little about the details of the molecular mechanisms of wound healing.

Infrared radiation transfers energy to surrounding tissues and is perceived as heat by thermoreceptors in the adjacent skin [10]. Infrared radiation has a longer wavelength than visible light and can be divided into FIR (5.6–1000 µm), middle-infrared radiation (1.5–5.6 µm), and near-infrared radiation (0.8–1.5 µm) [9]. Recent studies have shown that FIR therapy plays a beneficial role in the cardiovascular system [11]. FIR radiation improves endothelial function in patients with heart disease and increases access flow and patency of arteriovenous fistulas in hemodialysis patients [12–14]. Besides, FIR treatment can promote angiogenesis and microvascular blood flow in various animal models [11,15,16].

The present study aimed to test the hypothesis that FIR is effective in promoting diabetic wound healing. Therefore, this work aims to investigate the biological effect of FIR irradiation and the molecular mechanism of FIR on the healing of chronic wounds using the STZ-induced type I diabetes rodent wound model [17–19].

2. Materials and Methods

2.1. Animal Model

Four-month-old male Wistar rats were purchased from National Experimental Animals Production Center (Taipei, Taiwan). All experimental procedures and protocols involving animals were approved by the Institutional Animal Care and Use Committee on the protection of animals used for scientific purposes (IACUCA animal use protocol approval number: 107072) and in compliance with the Guide for the Care and Use of Laboratory Animals published by the National Institute of Health. Rats were housed in a 12-h light/dark cycle facility with a controlled temperature and kept with free access to water and food. A single dose of streptozotocin (STZ, 65 mg/kg, intraperitoneal injection, i.p.) in 0.1 M sodium citrate buffer was used in rats weighing 400 to 425 g, to generate a type I diabetic model according to previous reports [5,18,20]. The blood glucose levels from the tails were evaluated 1 and 2 weeks using a glucometer following STZ injection. Rats with glycemia more than 300 mg/dL were considered diabetic developed and then included for the following experiments. At two weeks after STZ induction, the rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and then subjected to full-thickness wounds. The skin flap tissue of the dorsum of the Wistar rats was excised to create a full-thickness skin defect with an area of $6 \times 5$ cm$^2$ wound defect, which has been described previously [20]. The wound was then temporarily covered with transparent Tegaderm (3M HealthCare, Borken, Germany) until FIR therapy was initiated. After surgery, the rats were returned to their cages in the animal holding room after they had regained consciousness. The rats were housed separately postoperatively.

2.2. Experimental Design

Male Wistar rats were divided into four groups (eight rats per group). Anesthesia was administered via inhalational general anesthesia of isoflurane along with an intramuscular injection of atropine (0.1 mg/kg) to reduce the secretion of saliva during and after the
surgery. The rats were randomized into four treatment groups: (1) Sham, non-diabetic control; (2) DM control, diabetic control without treatment; (3) FIR-20, diabetic rats received 20 min FIR (the rats were wounded as described and then exposed to lamps for 20 min per session, triplicate in a week for 4 weeks) and (4) FIR-40, diabetic rats received 40 min FIR (the rats were wounded as described and then exposed to lamps for 40 min per session, triplicate in a week for 4 weeks). The rats were exposed to radiation from a WSTM TY301 FIR emitter (Far IR Medical Technology, Taipei, Taiwan) with an effective energy intensity of 0.13 mW/cm². The wavelength of the light generated from the electrified ceramic plates was from 5 to 12 µm with a peak at 8.2 µm [1,10]. The radiator was set at a height of 20 cm above the cages for the indicated times (20 or 40 min).

2.3. Monitoring of Blood Perfusion in Wound Area Using Laser Doppler
The blood perfusion in the wound area was measured using a peripheral microvascular laser Doppler flow-perfusion imager (Lisca-PIMII, Linköping, Sweden). The blood perfusion in the wound area was detected on days 4, 8, and 15 during the first two sessions of FIR. Laser Doppler flowmetry was conducted using a camera-like device intended for two-dimensional mapping (imaging mode) and continuous recording (perfusion monitor mode) of superficial tissue blood perfusion. In the imaging mode, the low-power (1-mW) laser beam at a 670 nm wavelength via an optic fiber successfully scanned the tissue step-wise throughout several thousand measurement points. In tissue, the light is scattered and the frequency is shifted for interaction with moving blood cells based on the well-known Doppler principle. The sample depth was a few hundred micrometers. A fraction of the back-scattered and Doppler-broadened light was detected by a photo-detector in the scanner head. For each measurement point, the Doppler broadening and magnitude of the Doppler signal were calculated and a signal was generated that scales linearly with tissue perfusion defined as the product of red blood cell velocity and concentration. The results are presented as a two-dimensional color image on a computer monitor. The signal cannot be calibrated to an absolute value for blood flow; the output signal is therefore expressed as an arbitrary unit.

2.4. Wound Healing Observation
On the 0, 14th, 28th, and 42nd days after treatment, the changes in wounds and granulation tissue growth were observed with naked eyes. The wound healing area was assessed once a week after the operation using the template technique, which has been described previously [20]. Meanwhile, all wounds were photographed with a digital camera (COOLPIX B700, Nikon, Tokyo, Japan). By macroscopic observation, a distinct demarcation line in the unhealed tissue area was traced onto the transparent graph paper. The traced area was cut and measured its weight, and calculated by the formula \((1 - A1/A0) \times 100\%\), where \(A0\) is the original wound area \((6 \times 5 \text{ cm}^2)\) and \(A1\) is the unhealed area. The area was calculated once a week until the whole wound had healed [21].

2.5. Histological Examination
Histopathological observation of wound tissue structure on wound surface in the wound healing process was performed by HE staining experiment. Full-thickness 3-mm biopsies were obtained from the wound margin to investigate the pathological changes occurring in the wound at 1.5 weeks and 2.5 weeks post-treatment. Biopsy specimens were fixed in 10% formalin (Sigma-Aldrich, St. Louis, MO, USA) and embedded in paraffin. Sections for each group were stained with hematoxylin and eosin (H&E; Sigma-Aldrich). IHC semiquantitative staining was performed using horseradish peroxidase–diaminobenzidine (HRP–DAB) staining kit (R&D, Inc., Minneapolis, MN, USA) as described previously [18,22]. Polyclonal antibodies against CD45, VEGF, eNOS, EGF, 8-hydroxy-2-deoxyguanosine (8-OHdG), and Ki-67 (Santa Cruz, Santa Cruz, CA, USA) were used as the primary antibodies, and the sections were incubated with these antibodies at 1:100 dilutions in phosphate-buffered saline for one hour. The sections were then incubated with biotinylated goat
anti-rabbit antibodies for 30 min. The specific binding of the secondary antibodies to the primary antibodies was visualized using HRP for the enzymatic conversion of the chromogenic substrate 3,3′-diaminobenzidine (DAB) into a brown precipitate. The sections were mounted, cleared, coverslipped, and examined using a Zeiss fluorescence microscope (Carl Zeiss, Gottingen, Germany).

2.6. Examination of Histomorphometric Markers

Tissue sections were imaged using a Zeiss Axioskop 2 plus microscope (Carl Zeiss) to quantify the immunohistochemically stained cells. All images of each specimen were captured using a Cool CCD camera (SNAP-Pro cf. Digital kit; Media Cybernetics, Silver Spring, MD, USA). Images were analyzed using Image-Pro Plus image analysis software (Media Cybernetics) as described previously. Four random images from each selected area were acquired at 400 × magnification. The number of immunopositive cells and the percentage of positively labeled cells to total cells are presented.

2.7. Data Management and Statistical Analysis

The experimental results are presented as the mean ± standard deviation (SD). Significant differences between experimental groups with replicates in each of the three independent experiments were analyzed by using the t-test, and \( p < 0.05 \) was considered statistically significant.

3. Results

3.1. FIR in Enhancing Angiogenesis by Increase of Per-Wounding Blood Perfusion

A previous study showed that FIR, independent of its thermal effects, enhances blood perfusion indicating that FIR irradiation could regulate angiogenesis. The blood perfusion in the wound area was detected by laser Doppler after the FIR treatment. A Doppler signal was generated as red blood cell velocity expressed as an arbitrary unit and correlated to wound area tissue perfusion. The initial blood flow of FIR 40 groups is 9.29% more than FIR 20 on D8, 29.6% more than FIR 20 on Day 15. On Day 8, blood flow increased 70% after 40 min FIR treatment. The blood perfusion in the wound area revealed no significant difference between FIR-20 and FIR-40 groups on day 4 after FIR. However, the FIR-40 FIR-treated group still showed a significant increase in wound area perfusion on Day 8 after the FIR compared to FIR-20 FIR-treated diabetic rats (Figure 1B). These results indicated that FIR increases topical blood perfusion and facilitates the process of chronic wound healing.

3.2. FIR Enhanced Diabetic Wound Healing

The in vivo experimental results revealed that the wound size was significantly reduced during the wound healing process in the FIR-treated rats compared to the diabetic control rats. On the 0, 14th, 28th, and 42nd days after treatment, the changes in wounds and granulation tissue growth were observed and wound sizes were significantly reduced on the 28th and 42nd days post-treatment in the FIR-20 and FIR-40 groups compared with diabetic control (Figure 2A, \( p < 0.005 \)). The complete wound healing time was significantly faster in the FIR-20 and FIR-40 groups than in the diabetic control group without treatment (\( p < 0.001 \)) (Figure 2B). Nonetheless, the wound healing time in the FIR-20-treated group was still significantly longer than that in the FIR-40-treated rats (7.14 ± 0.90 weeks vs. 5.83 ± 1.08 weeks, \( p = 0.025 \)). This result indicated that treatment with the optimal time length of FIR could promote faster diabetic wound healing.
Figure 1. Far-infrared radiation (FIR) treatment in enhancing blood perfusion in the diabetic wound area. The blood perfusion in the wound area was detected by laser Doppler before (pre-FIR) and 1 h after the FIR (post-FIR) at the early and the late stage of treatment. (A) The initial blood flow in wounds was 365.38 ± 132.14 PU/wound for FIR 20 and 308.25 ± 82.99 PU/wound for FIR 40 on Day 4, which was set as 100%. (B) Upper: Laser Doppler Imager before (Pre-FIR) and after FIR treatment (Post-FIR). Lower: The change of blood flow was calculated by the expression (PostFIR – PreFIR)/PreFIR × 100% after FIR 20 and 40 min treatment respectively. The FIR-40 group showed a significant increase in wound area perfusion at the late phase after the FIR, *\( p < 0.05 \).

Figure 2. Far-infrared radiation (FIR) in accelerating wound healing in diabetic rats. (A) Wound size difference. Compare with DM, *\( p < 0.05 \); FIR-20 vs. FIR-40, #\( p < 0.05 \). (B) Average complete healing time. The complete wound healing time was significantly faster in the FIR group with 20 min (FIR-20) and 40 min (FIR-40) than the diabetic control group without treatment (\( p < 0.001 \)). Nonetheless, the wound healing time in the FIR-20-treated group was still significantly longer than that in the FIR-40-treated rats with two sessions (\( p = 0.025 \)).

3.3. FIR Suppressed the Inflammatory Response

The biopsy specimens retrieved from the wound edge area were histologically examined after the wounding. Hematoxylin and eosin staining revealed that leukocyte infiltration from the dermis to the subcutaneous muscular layers was markedly reduced in
the FIR-treated diabetic rat groups at 1.5 weeks and 2.5 weeks post-treatment compared to that in the diabetic controls (Figure 3A). The IHC staining indicated that CD45+ expression was significantly decreased in the FIR-20- and FIR-40-treated diabetic rats groups when compared with rats in the DM control group (Figure 3B). This indicated diabetic rats with FIR treatment could regulate the adequate inflammatory response.

**Figure 3.** Far-infrared radiation (FIR) treatment suppressed the early inflammatory responses and reduced CD45+ cells in periwound edges. (A) The specimens harvested from the periwound area were histologically examined. H&E staining revealed that leukocyte infiltration (black arrows) from the dermis to the subcutaneous muscular layers was markedly reduced in the FIR-20-treated and FIR-40-treated diabetic rat groups at the early phase (1.5 weeks) of post-treatment compared to that in the diabetic controls and consistently decreased at the late phase (2.5 weeks) after treatment among the FIR-treated groups and the diabetic control group without treatments. (B) IHC staining indicated that CD45+ expression (red arrows) was significantly decreased in the FIR-20- and FIR-40-treated groups at the early (1.5 weeks) and late-phase (2.5 weeks) after treatment among the FIR-treated groups and the DM control group without treatments. Summary of CD45+ IHC staining in periwound edges after FIR-treatment. Compare with DM, *p < 0.05 (at 1.5 weeks); #p < 0.05 (at 2.5 weeks). Magnification, 100×.

### 3.4. FIR Suppressed Oxidative Damage

The oxidative damage indicated by the levels of 8-OHdG revealed a significant decrease in the diabetic wound-healing process in the FIR-treated diabetic groups at 1.5 weeks and 2.5 weeks post-treatment compared to the diabetic control group (Figure 4). Furthermore, the rats in the FIR-40 group exhibited a marked decrease in 8-OHdG expression compared with the rats in the FIR-20 group.

### 3.5. FIR in Enhancing Cellular Proliferation and Regeneration

Cellular proliferation was analyzed in terms of the Ki-67 expression levels in the wound edge, which were determined by performing HRP-DAB IHC staining. The staining results revealed a significant increase in Ki-67 expression (Figure 5), especially in the fibroblasts in the basal epidermal and subcutaneous layers, in the FIR-20 and FIR-40, FIR-treated diabetic rats at 1.5 weeks and 2.5 weeks post-treatment compared with the rats in the diabetic control group. Furthermore, the rats in the FIR-40 FIR-treated diabetic group exhibited a marked increase in Ki-67 expression in the wound edge compared to that in the FIR-20 FIR-treated group. This finding indicated that the long time length of FIR could increase cellular proliferation earlier and promote wound healing processing.
Figure 4. Far-infrared radiation (FIR) treatment for suppression of oxidative stress. (A) The oxidative damage indicated by the 8-OHdG level (red arrows) revealed a significant decrease in the diabetic wound healing process in the FIR-treated groups at the early (1.5 weeks) and the late (2.5 weeks) phase post-treatment compared to the DM control group. (B) Summary of 8-OHdG level in periwound edges after FIR-treatment. Furthermore, the rats in the FIR-40 group exhibited a marked decrease in 8-OHdG expression compared with the rats in the FIR-20 group. * $p < 0.05$ (at 1.5 weeks); # $p < 0.05$ (at 2.5 weeks). Magnification, 400×.

Figure 5. Far-infrared radiation (FIR) treatment in facilitating cell proliferation. (A) Cellular proliferation was analyzed in terms of the Ki-67 expression levels (red arrows) in the wound edge, which were determined by performing HRP-DAB IHC staining. The staining results revealed a significant increase in Ki-67 expression, especially in fibroblasts in the basal epidermal and subcutaneous layers, in the FIR-20- and FIR-40-treated diabetic rat groups at the early (1.5 weeks) and the late (2.5 weeks) stage after treatment compared with the DM control group ($p < 0.001$). (B) Summary of Ki-67+ cells in periwound edges after FIR-treatment. Furthermore, the rats in the FIR-40 group exhibited a marked increase in Ki-67 expression in the wound edge compared to that in the FIR-20 group. * $p < 0.05$ (at 1.5 weeks); # $p < 0.05$ (at 2.5 weeks). Magnification, 400×.
3.6. FIR in Enhancing Angiogenesis by Upregulation of eNOS, VEGF and EGF Expression

During wound healing, angiogenic capillary sprouts invade the fibrin/fibronectin-rich wound clot and within a few days organize into a microvascular network throughout the granulation tissue. The angiogenic effect detected by eNOS, VEGF, and EGF expression in the periwounded tissue was investigated by using IHC staining. The eNOS, VEGF, and EGF levels were increased, particularly in fibroblasts and endothelial cells, in the FIR-20 and FIR-40 FIR-treated diabetic groups at 1.5 weeks and 2.5 weeks post-treatment compared to the diabetic control group (Figure 6). In addition, the eNOS, VEGF, and EGF expression levels along the wound edge were significantly increased in the FIR-40-treated diabetic rats at 1.5 weeks and 2.5 weeks compared with the FIR-20-treated diabetic group. These results indicated that the long time length of the FIR treatment group showed an enhanced angiogenic effect compared to the diabetic control group.

![Figure 6. Far-infrared radiation (FIR) treatment in facilitating angiogenesis. The angiogenesis effect detected by eNOS (A), VEGF (B), and EGF (C) expression in the peri-wound tissue was investigated by using IHC staining. Expression of eNOS, VEGF, and EGF levels were increased, particularly in fibroblasts and endothelial cells, in the FIR-20- and FIR-40-treated diabetic groups at the early (day 10) and the late (day 17) stage post-treatment compared to the diabetic control group. In addition, eNOS, VEGF, and EGF expression were significantly increased in the FIR-40 group at the early and the late stage after treatment compared with the FIR-20 group. * p < 0.05 (at 1.5 weeks); # p < 0.05 (at 2.5 weeks).](image)

4. Discussion

Unhealed chronic wounds are characterized by a prolonged inflammatory phase, delayed cellular proliferation, poor re-epithelialization, and impaired angiogenesis [3,23]. Since wound healing is impaired in diabetic patients, previous studies have reported various modalities, however, controversial results concerning wound healing in diabetic patients were found. Therefore, researchers have attempted to design effective interventions that could accelerate the wound healing process. More and more physical therapies are used for enhancing the process of wound healing, such as extracorporeal shock-wave therapy, negative pressure wound therapy, etc. [5,18]. Studies have revealed that FIR therapy improves circulation, vessel endothelial function, and reduces atherosclerosis among diabetic patients undergoing hemodialysis [14].

The present study indicates that FIR therapy at least has two biological effects on chronic wound healing, including reduced inflammation and oxidative damage as well as promotion of proliferation and angiogenesis. Studies have shown that leukocyte-mediated inflammation is an important factor during the wound-healing process [20,24,25]. Histological analysis of the wound margin showed that the infiltration of inflammatory cells was attenuated on day 10 after the low strength and high strength FIR-treated rats compared with the diabetic controls. A previous study had shown that oxygen radicals were significantly increased in diabetic rats [17]. The expression of 8-OHdG was also markedly increased in the diabetic endothelium and subintima compared to that in normal vessels [17]. To assess the oxidative change of chronic wounds, we performed IHC staining of 8-OHdG. The results revealed that the level of 8-OHdG was significantly decreased in the low strength and high strength FIR-treated groups during the diabetic wound healing process.
process. Furthermore, the rats in the high strength FIR-treated group exhibited a marked
decrease in 8-OHdG expression compared to that in the low strength FIR-treated diabetic rats. These results indicated that high strength FIR treatment more effectively and signifi-
cantly suppressed 8-OHdG expression than low strength. This may be considered a
further validation of reduced inflammatory responses in FIR-treated rats compared with
the diabetic controls. Further studies should be needed to provide more semi-quantification
data analysis, such as TNFα or IL-2 expressions, to elucidate the inflammatory-related
pathways in FIR facilitating wound healing.

Cellular proliferation and regeneration were examined in terms of Ki-67 expression in
the wound margin [4,20]. The cell proliferation marker Ki 67 is expressed by proliferating
cells in the late G1, S, and G2/M phases of the cell cycle. The nuclear localization of Ki 67
and its specific association with the cell cycle shows its significance in the regulation of cell
division during the wound proliferation phase. The evidence from this study implied that
whether FIR-20 or FIR-40 FIR-treated groups had a marked increase in Ki-67 expression,
particularly in the fibroblasts and basal epidermal layers. Furthermore, the rats with
FIR-40-treated diabetic rats exhibited a marked increase in Ki-67 expression in the wound
margin compared to the rats in the FIR-20-treated group. This finding indicated that the
long time length of the FIR treatment was more effective in increasing cellular proliferation
and facilitating the process of chronic wound healing.

Angiogenic factors play an important role in the wound healing process. ENOS is an
activated enzyme required for angiogenesis, and VEGF is recognized as the most important
biological indicator of angiogenesis; EGF is also considered to be an important growth
factor for important vascular endothelial cells in addition to being related to angiogenesis.
In the present study, expression of eNOS, VEGF, and EGF in the wound edge after treatment
was investigated by using IHC staining. The experimental results indicated that expression
of angiogenic factors, eNOS, VEGF, and EGF was significantly elevated in the wound
margin area, particularly in endothelial cells and fibroblasts, at 1.5 weeks and 2.5 weeks
after the low strength or high strength FIR treatment compared with the diabetic control
group. Moreover, the eNOS, VEGF, and EGF expression levels were increased in the FIR-40-
treated group compared with the FIR-20 treatment group. This result demonstrated that the
long time length of the FIR treatment was more effective in enhancing angiogenesis and the
induction of neovascularization in the transitional zone of the wound margin. Additionally,
blood circulation detected by a laser Doppler flow-perfusion imager showed no significant
differences existed in early tissue perfusion in FIR-20- or FIR-40-treated groups. However,
a significant increase existed in late tissue perfusion between the FIR-20-treated group and
the FIR-40-treated diabetic rats. This finding indicates that FIR-40 has late enhanced topical
blood perfusion and facilitates the wound healing process. This demonstrates that FIR
could significantly increase blood perfusion and epithelialization during wound healing in
a model of diabetes.

However, there are still some limitations to this study. This study is limited by the
small number of animals, thereby resulting in a relatively low statistical power. Addition-
ally, this is a very early-stage study concerning the comparison of low strength and
high strength FIR for wound healing in a rodent model of diabetes. Many additional
experiments are required to overcome the limitations of our experimental design to eluci-
date the mechanical effects such as the inflammatory activity of systemic effects detected
by flow cytometry, or using ELISA, as well as more wound healing-related molecules of
per-wounding tissue expressions such as HIF-1, angiogenesis-related pathway [25].

In summary, this study demonstrated that both time lengths of FIR enhanced diabetic
wound healing by their effects on wound epithelium, suppression of the inflammatory
response, facilitation of cellular proliferation, and effects on angiogenesis and oxidative
damage. However, the optimal time length of the FIR treatment was more effective in
the acceleration of wound healing than the diabetic control. This technique represents
a feasible method for accelerating wound healing or augmenting compromised tissue
circulation and diabetic ulcers.
5. Conclusions

Our results have revealed that both time lengths of FIR enhanced diabetic wound healing by their effects on wound epithelium, suppression of the inflammatory response, facilitation of cellular proliferation, and effects on angiogenesis and oxidative damage (Figure 7). However, the optimal time length of the FIR treatment was more effective in the acceleration of wound healing than the diabetic control. This technique represents a feasible method for accelerating wound healing or augmenting compromised tissue circulation and diabetic ulcers.

Figure 7. Proposed the biomechanisms of far-infrared radiation (FIR) treatment suppress the leukocyte infiltration, decreasing inflammatory response (e.g., CD45+, 8-OHdG), enhancing cellular proliferation (e.g., Ki-67+ cells) and angiogenic factors (e.g., eNOS, VEGF, EGF), resulting in inducing epithelialization and wound healing.

Author Contributions: R.-F.C. and K.-F.L. participated in the study design and the acquisition of data. S.-S.L., S.-H.H. and Y.-C.W. participated in the analysis and interpretation of the data. Y.-R.K. and R.-F.C. participated in the data analysis and the drafting of the manuscript and/or its critical revision. C.-T.W. participated in the acquisition of animal data. Y.-N.L. and Y.-R.K. participated in the research design and revision of the manuscript. All authors have read and agreed to the published version of the manuscript.

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References
1. Hsu, Y.-H.; Lin, Y.-F.; Chen, C.-H.; Chiu, Y.-J.; Chiu, H.-W. Far infrared promotes wound healing through activation of Notch1 signaling. J. Mol. Med. 2017, 95, 1203–1213. [CrossRef] [PubMed]
