# New Insights in Diabetic Foot Ulcer Mechanisms: DNA Methylation and Non-coding RNAs

*Novas Perspetivas dos Mecanismos da Úlcera do Pé Diabético: Metilação do ADN e ARNs Não-codificantes*

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## Abstract

Diabetic foot ulcers (DFUs) are considered a serious complication of diabetes with complex pathogenic factors. Normal tissue repair proceeds through well-coordinated overlapping phases including hemostasis, inflammation, proliferation, and remodeling. In diabetes, the progression through these phases is impaired resulting in a sustained inflammatory state and delayed wound healing. Epigenetic mechanisms of gene regulation, such as DNA methylation and non-coding RNAs, are associated with the synchronized regulation of the wound healing process involving multiple cell types. The epigenetic regulation is sensitive to chronic hyperglycemia and is known to cause pathogenesis of microvascular complications, such as DFU. Several studies suggest that altered epigenetic regulation in skin cells from wounds influence cell phenotypes and the healing progression, particularly in pathologic states such as diabetes. In this review, we discuss the epigenetic mechanisms of DNA methylation and regulation of non-coding RNAs in tissue repair and highlight recent findings that demonstrate how epigenetic events are altered through the progression of diabetic wound healing. Dissecting the dynamic interplay between cellular subtypes involved in wound healing and epigenetic mechanisms will strengthen our understanding of how to improve the healing outcomes in patients affected by DFUs.

**Keywords:** diabetic foot ulcer; epigenetic mechanisms; wound healing; skin

### *Resumo*

As úlceras do pé diabético (UPD) são consideradas uma complicação grave da diabetes com fatores patogénicos complexos. A normal reparação do tecido ocorre através de fases sobrepostas bem coordenadas, incluindo a hemóstase, inflamação, proliferação e remodelação. Em condições de diabetes, a progressão das fases da cura da ferida é disfuncional, resultando num estado de inflamação crónico e na cicatrização demorada de feridas. Mecanismos epigenéticos de regulação génica, como a metilação do ADN e ARNs não codificantes, estão associados à regulação sincronizada do processo de cicatrização de feridas envolvendo múltiplos tipos de células. A regulação epigenética é influenciada pela hiperglicemia crónica e é conhecida causar patogénese das complicações microvasculares, como as UPDs. Vários estudos sugerem que a alteração da regulação epigenética das células da pele em feridas influencia os fenótipos celulares e a progressão da cicatrização, particularmente em estados patológicos como a diabetes. Nesta revisão, discutimos os mecanismos epigenéticos de metilação do ADN e regulação de ARNs não codificantes na cicatrização do tecido. Destacamos as descobertas recentes que demonstram como os eventos epigenéticos estão alterados na progressão da cicatrização de feridas diabéticas. Dissecar a interação dinâmica entre os subtipos celulares envolvidos na cicatrização de feridas e nos mecanismos epigenéticos reforçará a nossa compreensão de como melhorar os resultados de cicatrização em doentes com UPDs.

**Palavras-chave:** úlcera do pé diabético; mecanismos epigenéticos; cicatrização da ferida; pele

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## > INTRODUCTION

Diabetic foot ulcers (DFU) are a serious complication of diabetes, with a prevalence of 15–25 % among patients with diabetes, [1] and cause higher economic burden and mortality. [2] Approximately 50–60% of patients with DFUs will develop diabetic foot infection (DFI) and 15% will undergo amputation. Furthermore, the 5-year risk of death in patients with DFUs is 2.5 times higher than in patients without foot ulcers. [3] Taken this into account, it is key to focus on the study of DFU mechanisms to develop better treatments and improve patient well-being.

Cutaneous wound healing is a complex and highly coordinated process involving numerous cell types to accomplish four overlapping phases of hemostasis, inflammation, proliferation, and tissue remodeling. [4] In the hemostasis phase, immediately after injury, platelets activate to produce fibrin clots, and immune cells are recruited to the wound areas. In the inflammatory phase, neutrophils are initially involved to clear bacteria, together with macrophages that exert early pro-inflammatory and late anti-inflammatory functions during the healing process. The proliferation stage is characterized by the formation of new blood vessels, while fibroblasts deposit and remodel the extracellular matrix (ECM), and keratinocytes proliferate and migrate to close wounds. During the remodeling phase, cells in the granulation tissue undergo apoptosis, and macrophages break down excessive ECM and apoptotic cells. [4, 5]

Epigenetics refers to heritable changes in gene expression without altering the nucleotide sequence and includes three main mechanisms: DNA methylation, regulation through non-coding RNAs (ncRNAs), and histone modification. [6] Epigenetic regulation is essential for the highly coordinated processes of wound healing. [7] Dysregulation of epigenetic mechanisms caused by diabetes contributes to poor wound healing. Also, it is likely that epigenetic alterations are related with the adverse effects of diabetic complications that persist for a long time even after hyperglycemia is controlled to ideal levels, thus only long-term intensive glycemic control can mitigate the risk of developing diabetic complications. [8, 9]

This review highlights the epigenetic mechanisms in diabetic wound healing, particularly DNA methylation and regulation through non-coding RNAs (ncRNAs) represented by microRNA (miRNA), long non-coding RNA (lncRNA), and circular RNA (circRNA), in pre-clinical and clinical studies, to valuable insights into the pathophysiology of DFUs.

## > MECHANISMS OF DNA METHYLATION IN DIABETIC WOUND HEALING

DNA methylation, in mammals, is catalyzed by enzymes of the DNA methyltransferase (DNMT) family that transfer a methyl group to the C5 position of cytosine in CpG dinucleotides (cytosine 5' to a guanine, separated by a phosphodiester bond). [10] DNA methyltransferase-1 (DNMT1), DNMT3A, and DNMT3B are the main methyltransferase enzymes involved in DNA methylation. Also, the ten-eleven translocation (TET) family is responsible for DNA demethylation. [11]

DNA methylation and demethylation are known to regulate diabetic wound healing, in several aspects of wound repair such as inflammation, proliferation and migration, angiogenesis and collagen deposition. [12-15] Thus, we focus here on the roles of DNA methylation in diabetic wound healing (Table 1).

## > DNA METHYLATION IN INFLAMMATION

Chronic inflammation in diabetic wounds is a key contributor to poor wound healing. In the early inflammatory phase, macrophages are predominantly in the pro-inflammatory M1-like phenotype, and then polarized to the regenerative M2-like phenotype in the transition from late inflammatory phase to proliferative phase. [16] However, under diabetic conditions, the macrophage phenotype polarization is severely compromised, leading to impaired wound healing. [17, 18]

In type 2 diabetic db/db$^{-/-}$ mice, bone marrow-derived stem cells have shown increased levels of DNMT1 and a pro-inflammatory M1-like macrophage phenotype. [12, 19] Moreover, wound macrophages of diet-induced obese and db/db$^{-/-}$ mice showed an inhibition of DNMTs 3a and 3b. [20] This effect led to a hypomethylation and overexpression of ciclo-oxigenase-2 (Cox-2) promoting an increase in prostaglandin E2 (PGE2) synthesis. The increase in the activation of Cox-2/PGE2 pathway caused an increase in macrophage inflammatory cytokine expression and impaired phagocytosis of bacteria. [20]

The toll-like receptor 2 (TLR2) promoter was found methylated at CpG sites in wounds of patients with DFU. [21] The upregulation of DNMT1 expression and consequent hypermethylation of the neurogenic locus notch homolog protein 1 (Notch1), transcription factor PU.1, and Krüppel-like factor 4 (Klf4), were shown to cause poor wound healing in diabetic db/db$^{-/-}$ mice through the dysregulation of hematopoietic stem cells (HSCs) towards macrophage differentiation. [19] This inflammatory mechanism has shown that diabetes induced epigenetic al-

**Table I** - The role of DNA methylation mechanisms in diabetic wound healing.

| Factor | Target | Methylation | Condition | Impact | Ref. |
|---|---|---|---|---|---|
| DNMT1 | - | Methylation (increase) | Marrow-derived stem cells from diabetic mice | Pro-inflammatory macrophage phenotype | (12, 19) |
| DNMT 3a and 3b | COX-2 | Methylation (decrease) | Wound macrophages of diet-induced obese and db/db$^{-/-}$ mice | Increase in macrophage inflammatory cytokine expression and impaired phagocytosis of bacteria | (20) |
| - | TLR2 promoter | Methylation (increase) | DFUs | Induction of innate immune and inflammation response | (21) |
| DNMT | NET components | Methylation (decrease) | DFUs | Increase in spontaneous NETosis, but impaired inducible NETosis, consequently delaying healing | (22) |
| DNMT1 | Ang-1 | Methylation (increase) | Human umbilical vein endothelial cells cultured in high glucose conditions | Persistent activation of NF-κB and subsequent endothelial dysfunction | (29) |
| DNMT1 | Flt-1 promoter | Methylation (increase) | Rat mesenchymal stem cells | Differentiation of mesenchymal stem cells to endothelial cells | (30) |
| TET2 | MMP-9 promoter | Methylation (decrease) | Human primary keratinocytes | Increase in MMP-9 expression, leading to impaired healing | (33,34) |

Legend: Ang – angiopoietin; COX - ciclo-oxigenase-2; DFU – diabetic foot ulcer; DNMT - DNA methyltransferase; Flt - vascular endothelial growth factor (VEGF) receptor 1; MMP - matrix metalloproteinase; NET - neutrophil extracellular traps; NF-κB - nuclear factor-κB; TET - ten-eleven translocation; TLR - toll-like receptor.

terations in HSCs, which in turn determined the gene expression of terminally differentiated inflammatory cells. DNA methylation also affects neutrophil function. Neutrophils are important for wound healing as they release neutrophil extracellular traps (NETs) and subsequent death by NETosis, which is crucial for their antimicrobial function. This process is dysregulated in wounds of db/db$^{-/-}$ mice contributing to delayed wound healing. [22] Furthermore, DNMT inhibition caused increased NETosis. [23] Taken together, these findings suggest that DNA methylation may be an important epigenetic regulator in the inflammatory phase of wound healing via modulation of neutrophils and macrophages functions.

### > DNA METHYLATION IN ANGIOGENESIS

Angiogenesis is impaired in diabetic wound healing. DNMT1 plays a key role in angiogenesis, and several studies have shown that inhibition of DNMT1 expression is beneficial for angiogenesis. [24, 25]
Angiopoietin-1 (Ang-1) and angiopoietin-2 (Ang-2) promote the formation of mature and functional microvessels and maintain endothelial integrity, which is essential for angiogenesis. [26] However, Ang-1 levels are decreased in wounds of diabetic mice. [27, 28] Transient hyperglycemia increases DNMT1 expression in endothelial cells and in turn it leads to Ang-1 hypermethylation and decreased Ang-1 expression resulting in the persistent activation of nuclear factor-κB (NF-κB) and subsequent endothelial dysfunction. [29] Furthermore, inhibi-

tion of DNMT1 promotes angiogenesis and wound healing via regulation of Ang-1/NF-κB signaling pathway. [29] Moreover, nitric oxide (NO), important for wound healing, promotes the differentiation of mesenchymal stem cells to endothelial cells by inhibiting DNMT1 expression and consequently the methylation of the vascular endothelial growth factor (VEGF) receptor 1 (Flt-1) promotor. [30] These results emphasize the long-term effects that hyperglycemia-induced DNA methylation has on poor wound healing, suggesting that targeting DNMT1 has therapeutic potential in diabetic wound healing.

### > DNA METHYLATION IN FIBROBLASTS AND KERATINOCYTES

DNA methylation and demethylation occurs in fibroblasts and keratinocytes in diabetic wound healing. Fibroblasts from non-healing DFUs show lower global DNA methylation and functional annotation identified enrichment of genes associated with angiogenesis and extracellular matrix (ECM) assembly. [31]
In patients with diabetes, the matrix metalloproteinase-9 (MMP-9), a type IV collagenase important in cell migration and remodeling, is highly expressed in keratinocytes at the wound site, leading to impaired epithelialization and poor healing. [32] In human primary keratinocytes, advanced glycosylation end products (AGEs) cause upregulation of TET2 gene expression, which leads to the increase in DNA demethylation in specific re-

gions of the MMP-9 promoter and increased the MMP-9 levels. [33] Furthermore, under inflammatory conditions, keratinocytes showed site-specific DNA demethylation in the MMP-9 promoter, which was correlated with increased MMP-9 expression. [34] Altogether, this suggests that targeting DNA demethylation could be a possible therapeutic approach in chronic wounds associated with increased levels of MMP-9. [35, 36]

## > MECHANISMS OF NON-CODING RNAs IN DIABETIC WOUND HEALING MIRNA IN DIABETIC WOUND HEALING

MicroRNAs (miRNAs) are short, highly conserved non--coding RNA molecules (~22 bp) that regulate target gene expression at a post-transcriptional level. [37, 38] Most miRNAs bind to and interact with the 3' UTR of target mRNAs resulting in target gene silencing, [39, 40] The regulation of miRNAs is important in all phases of wound healing. [41-43] Thus, we focus here on the roles of miRNAs in diabetic wound healing (Table 2).

## > MiRNA IN INFLAMMATION

Inflammation is important to eliminate pathogens and remove dead tissue, but excessive or prolonged inflammation can lead to poor wound healing. MiRNAs have become important players in wound healing by regulating inflammatory signaling pathways and modulating the function of immune cells. [44]

The inflammatory pathway COX-2/PGE$_2$ was found increased in wound-derived macrophages of human and mice with diabetes via inhibition of DNMT 3b–mediated hypermethylation of the Cox-2 promoter by the up-regulation of miR-29b. [20] MiR-497 expression was reduced in skin wounds of diabetic mice, and the intradermal injection of miR-497 accelerated wound healing by downregulating pro-inflammatory cytokines, such as interleukin (IL)-1β, IL-6, and tumor necrosis factor-alpha (TNF-α). [45] Moreover, miR-146a expression was decreased in wounds of diabetic mice along with upregulated pro-inflammatory cytokine expression, whilst the increase in miR-146a expression promoted diabetic wound healing. [46] Moreover, the increase of miR-23b, with miR-23b mimic treatment, inhibited pro-inflammatory TNF-α, IL-1β, IL-6, and chemokine monocyte chemoattractant protein 2 (CCL2), and increased anti--inflammatory IL-10, decreasing the infiltration of inflammatory cells in the wounds of diabetic mice by targeting apoptotic signal-regulating kinase 1 (ASK1). [47]

The miR-17-92 cluster is a polycistronic miRNA, which produces seven mature miRNAs: miR-17-3p, miR-17-5p, miR-18a, miR-19a, miR-20a, miR-19b, and miR-92a. [48, 49] The miR-17-92 cluster was found upregulated during acute wound healing and downregulated in chronic DFUs. [50] The deletion of miR-17-92 cluster led to delayed wound closure in diabetic mice.

MiR-132 was found suppressed in DFUs and diabetic db/db$^{-/-}$ mice. [51] The treatment with miR-132 accelerated re--epithelialization of human *ex vivo* skin wounds, increased wound closure in mice and suppressed inflammation, in part through inhibition of the NF-κB signaling. [51]

Human wounds infected with *Staphylococcus aureus*, a common colonizer of DFU, triggered miR-15b-5p expression, where it suppresses DNA repair and the inflammatory response through downregulation of multiple target genes including IKBKB, WEE1, FGF2, RAD50, MSH2 and KIT. [52]

MiR-129-2-3p was found downregulated in bone marrow-derived neutrophils of db/db$^{-/-}$ diabetic mice. [53] Besides, miR-129-2-3p mimic treatment of wounds inhibited the genes encoding for caspase 6 (Casp6) and receptor for monocyte chemoattractant protein-1 (Ccr2) to regulate the function of neutrophils and promote diabetic wound healing. [53] Moreover, miR-155 was increased in diabetic mouse skin, which increased inflammatory cell infiltration and downregulated fibroblast growth factor 7 (FGF7), possibly leading to impaired proliferation and migration of keratinocytes or fibroblasts. In addition, miR-155 inhibition was found to promote wound healing in diabetic mice. [54]

Specific miRNAs may also represent good biomarkers for DFUs. Indeed, miR-191 and miR-200b upregulation was found in plasma samples from patients with diabetes and chronic wounds. The increased circulating levels of miR-191 and miR-200b correlated with inflammatory markers and chronic wound size. [55] Furthermore, the expression of miR-21-5p, miR-155-5p, miR-146a-5p, and miR-221-3p were increased in plasma samples of patients with DFUs and concomitant psychological distress when compared to patients with DFUs but without psychological distress. This was further correlated with an increase in the immune cell ratio between effector CD4$^+$ and CD8$^+$ T cells and naive CD4$^+$ and CD8$^+$ T-cells, which is also associated with poor wound healing. [56] Yet, the plasma levels of miR-146a-5p itself, from patients in the same cohort, suggested a good healing prognosis by decreasing inflammation. [57]

## > MiRNA IN ANGIOGENESIS

Hyperglycemia and endothelial dysfunction in diabetes

**Table II** - The role of non-coding RNAs in diabetic wound healing.

| ncRNA | Target | Condition | Impact | Ref. |
|---|---|---|---|---|
| **microRNA in diabetic wound healing** | | | | |
| miR-29b | DNMT 3b | Wound-derived macrophages of human and mice with diabetes | Increases inflammation via increase of COX-2/PGE2 pathway | (20) |
| miR-497 | IL-1β, IL-6, TNF-α | Wounds of diabetic mice | Increases inflammation | (45) |
| miR-146a | Pro-inflammatory cytokine | Wounds of diabetic mice | Increases inflammation | (46) |
| miR-23b | ASK1 | Wounds of diabetic mice | Inhibits inflammation | (47) |
| miR-17-92 cluster | - | DFUs; Wounds of diabetic mice | Delays wound closure | (48-50) |
| miR-132 | NF-κB signaling | DFUs; Human *ex vivo* skin wounds | Inhibits inflammation; Accelerates re-epithelialization | (51) |
| miR-15b-5p | IKBKB, WEE1, FGF2, RAD50, MSH2 and KIT | DFUs | Suppresses DNA repair and the inflammatory response | (52) |
| miR-129-2-3p | Casp6 and Ccr2 | Bone marrow-derived neutrophils of diabetic mice | Regulates the function of neutrophils and promotes diabetic wound healing | (53) |
| miR-155 | FGF7 | Skin of diabetic mouse | Increases inflammatory cell infiltration | (54) |
| miR-191, miR-200b | - | Plasma of patient with DFU | Increases inflammation; Inhibits angiogenesis | (55) |
| miR-21-5p, miR-155-5p, miR-146a-5p, miR-221-3p | - | Plasma of patients with DFUs | Increases the immune cell ratio CD4^{+} and CD8^{+} T-cells; Associates with poor wound healing | (56) |
| MiR-21-5p | VEGF | Hindlimb ischemia rat diabetic foot model | Promotes diabetic foot ischemic repair and angiogenesis | (63) |
| miR-23c | SDF-1α | Infected DFU | Inhibits angiogenesis | (64) |
| miR-26a | SMAD1 | Wounds of diabetic mice | Inhibits angiogenesis | (61) |
| miR-27b | TSP-1, Sema6A, and p66shc | Bone marrow-derived angiogenic cells of diabetic mice | Promotes angiogenesis | (62) |
| miR-195-5p, miR-205-5p | VEGF-A | Plasma samples from patients with DFUs; Wounds of diabetic mice | Inhibits angiogenesis and migration of endothelial cells | (64) |
| miR-21-5p, miR-34a-5p, miR-145-5p | - | Fibroblasts isolated from DFUs | Induces cell senescence, and impairs cell proliferation and migration | (65) |
| miR-21-3p | SPRY1 | Fibroblasts cultured under high glucose; Wounds of diabetic mice | Induces proliferation, collagen synthesis, and growth factor release | (66) |
| miR-210 | - | Wounds of diabetic mice | Increases proliferation and migration of keratinocytes | (67,68) |
| miR-129, miR-335 | SP1 | Serum and tissue samples from patients with DFUs; Diabetic rat model | Induces MMP9 expression and poor wound healing | (69) |
| **LncRNA in diabetic wound healing** | | | | |
| GAS5 | STAT1 | Wounds of diabetic mice | Induces macrophage polarization towards the M1-like phenotype | (79) |
| Lethe | NF-κB | Macrophages under high glucose | Increases ROS | (80) |
| H19 | PTEN | Mesenchymal stem cells-derived exosomes | Increase PI3K/AKT1 signaling pathway | (81) |
| WAKMAR1 | - | Human *ex vivo* wound model | Promotes migration of keratinocytes | (83) |
| MALAT1 | NFR2, HIF1, VEGF | Infected DFUs; Endothelial cells | Downregulates angiogenic factors | (85) |

*(continuação)*

| ncRNA | Target | Condition | Impact | Ref. |
|---|---|---|---|---|
| TETILA | MMP9 | Skin of diabetic patients | Induces MMP-9 expression and impairs healing | (86) |
| URIDS | PLOD1 | Skin of diabetic rat model | Decreases collagen deposition and impairs healing | (87) |
| **CircRNA in diabetic wound healing** | | | | |
| has_circ_0084443 | PI3K, EGFR and ERK | DFUs; Human keratinocytes | Decreases migration | (93) |
| hsa_circ_0000907, hsa_circ_0057362 | - | Serum and serum-derived exosomes | Early diagnosis of DFU | (35,36) |

Legend: AKT - protein kinase B; ASK - apoptotic signal-regulating kinase; Casp6 - caspase 6; Ccr2 - receptor for monocyte chemoattractant protein-1; COX - ciclo-oxige-nase-2; DFU – diabetic foot ulcer; DMNT - DNA methyltransferase; EGFR - epidermal growth factor receptor; ERK - extracellular signal-regulated kinase; FGF – fibroblast growth factor; HIF - hypoxia-inducible factor; IKBKB - inhibitor of nuclear factor kappa B kinase subunit beta; IL – interleukin; KIT – tyrosine-protein kinase KIT; MALAT1 - metastasis-associated lung adenocarcinoma transcript 1; MMP - matrix metalloproteinase; MSH2 - MutS homolog 2; NFR2 - nuclear factor erythroid 2-like 2; NF-κB - nuclear factor-κB; p66shc - Src homologue and collagen homologue (Shc) adaptor protein; PGE - prostaglandin E; PI3K - phosphoinositide 3-kinase; PLOD1 - Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1; PTEN - Phosphatase and Tensin Homolog; RAD50 – S. cerevisiae RAD50 homolog, gene encoding for protein involved in DNA double-strand break repair; ROS – reactive oxygen species; SDF - stromal derived factor; Sema6A - Semaphorin 6A; SMAD1 - SMAD Family Member 1; SP1 - specificity protein-1; SPRY1 - protein sprout homolog 1; STAT - signal transducer and activator of transcription; TETILA - TET2-interacting lncRNA; TNF - tumor necrosis factor; TSP-1 - Thrombospondin 1; VEGF - vascular endothelial growth factor; WAKMAR1 - wound and keratinocyte migration associated lncRNA 1; WEE1 – gene encoding for tyrosine kinase Ser/Thr family; URIDS - upregulated in diabetic skin.

can lead to impaired angiogenesis, which increase the risk for diabetic foot ulcers development. In DFU, various miRNAs were found associated with vascular function, angiogenesis, and angiogenesis-related gene transcription. [58]

The antagomir of miR-15b and miR-200b, applied in intradermally at the wound edge, increased the number of new blood vessels in wound of diabetic mice by upregulating VEGF, Ang-1, and its receptor. [59] In patients with infected DFU, the miRNA-23c expression was increased and correlated with decreased stromal derived factor-1 alpha (SDF- 1α), indicating that miR-23c might inhibit angiogenesis in diabetic wounds by specifically regulating SDF-1α. [60]

MiR-26a expression was increased in wounds of db/db$^{-/-}$ mice. Local inhibition of miR-26a induced angiogenesis by increasing SMAD Family Member 1 (SMAD1) signaling in endothelial cells, independently of altered M1/M2 macrophage ratios. [61] MiR-27b expression was decreased in bone marrow-derived angiogenic cells (BMACs) from diabetic mice, and miR-27b mimic promoted BMAC therapy on diabetic wound closure and rescued impaired BMAC angiogenic function by repressing TSP-1, Sema6A, and p66shc genes. [62]

The pro-angiogenic miR-21–5p promotes ischemic repair in a hindlimb ischemia rat diabetic foot model and angiogenesis by upregulating VEGF receptor and activating serine/threonine kinase (protein kinase B, AKT) and mitogen-activated protein kinase (MAPK). [63]

Both miR-191 and miR-200b were upregulated in plasma samples from patients with DFUs. Both miRNAs inhibited angiogenesis and migration of endothelial cells overexpressing these miRNAs. [55]

MiR-195–5p and miR-205–5p found in extracellular vesicles isolated from DFU fluid, inhibited angiogenesis and decreased wound healing in patients with diabetic foot by directly inhibiting VEGF-A. [64]

## > MiRNA IN FIBROBLASTS AND KERATINOCYTES

MiR-21-5p, miR-34a-5p and miR-145-5p were upregulated in fibroblasts isolated from DFUs, and together with the suppression of their targets contributed to cell senescence in DFU fibroblasts and impaired cell proliferation and migration. [65]

MiR-21-3p expression was suppressed in fibroblasts cultured under high glucose, whereas the miR-21-3p agonist regulated the reduction of the protein sprout homolog 1 (SPRY1) promoting fibroblast proliferation, collagen synthesis, and growth factor release and accelerating wound healing in diabetic mice. [66]

The decrease in miR-210 was associated with impaired proliferation and migration of keratinocytes which lead to poor wound healing in wounds of diabetic mice. [67] The local injection of miR-210 mimics increased granulation tissue, cellular proliferation, and angiogenesis in wounds of diabetic mice by inhibiting the oxygen consumption rate (OCR), enhancing glycolysis, and subsequently decreasing reactive oxygen species (ROS) levels restoring the metabolic balance. [68]

Downregulation of miR-129 and miR-335 was found in both serum and tissue samples from patients with DFUs.

Specificity protein-1 (SP1) gene, upregulated in DFUs, is a direct target of both miRNAs. SP1 binds directly to the MMP-9 promoter and induces its expression. The induced overexpression of miR-129 and miR-335 accelerated wound closure and downregulated SP1 and MMP-9 expression in a diabetic rat model. [69]

## > LncRNA IN DIABETIC WOUND HEALING

LncRNAs are defined as a type of ncRNA longer than 200 nucleotides not translated into proteins. [70, 71] LncRNAs are regulators of several cellular processes, including nuclear chromatin organization, mRNA stability, transcription, translation and cytoplasmic post-translational modifications. [72, 73] LncRNAs have established roles in the pathology of many diseases, including cancer, cutaneous disorders and diabetes. [70, 74-78] The LncRNAs involved in diabetic wound healing are indicated in Table 2.

LncRNA GAS5 was overexpressed in wounds of diabetic mice, which promoted macrophage polarization towards the M1-like phenotype by increasing the signal transducer and activator of transcription 1 (STAT1). [79] Additionally, the knockdown of lncRNA GAS5 expression, with lentiviral short hairpin RNA, promoted the transition from M1- to M2-like macrophages to rescue impaired wound healing in db/db$^{-/-}$ mice. [79] Moreover, high glucose conditions decreased lncRNA Lethe in macrophages, resulting in increased reactive oxygen species production via NF-κB signaling. [80]

Fibroblasts from patients with DFUs presented decreased expression of LncRNA H19. [81] Likewise, mesenchymal stem cells (MSC)-derived exosomes containing LncRNA H19 were shown to promote fibroblasts proliferation and migration, as well as decreased apoptosis and inflammation. LncRNA H19 was shown to bind to and suppress miR-152-3p, which in turn increased the level of its target gene Phosphatase and Tensin Homolog (PTEN) and therefore, the downstream activation of phosphoinositide 3-kinase/ protein kinase B (PI3K/AKT1) signaling. Moreover, the induced overexpression of lncRNA H19 in DFU fibroblasts reduced miR-29b levels, resulting in the upregulation of Fibrillin 1 (FBN1), enhancing the proliferation and migration of fibroblasts. [82] Thus, the delivery of lncRNA H19 could be used as an approach for improved fibroblast function under diabetic conditions. [81]

The wound and keratinocyte migration associated lncRNA 1 (WAKMAR1) was found suppressed in DFUs. [83] LncRNA WAKMAR1 inhibited methylation of the E2F transcription factor 1 (E2F1) promoter by sequestering DNMTs and promoted migration of keratinocytes and re-epi-

thelialization of human *ex vivo* wound model. [83] On the other hand, silencing of lncRNA WAKMAR2 inhibited inflammatory chemokine production of keratinocytes, decreased cell migration and impaired re-epithelialization in human *ex vivo* wounds. [84]

LncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) was decreased in infected DFUs, [85] and this correlated with downregulation of nuclear factor erythroid 2-like 2 (NRF2), hypoxia-inducible factor 1 (HIF1) and VEGF. Besides, the silencing of lncRNA MALAT1 in endothelial cells reduced the expression of pro-angiogenic factors HIF1 and VEGF, as well as pro-inflammatory TNF-α and IL-6. [85]

LncRNA TETILA (TET2-interacting lncRNA) was upregulated in human diabetic skin. LncRNA TETILA recruits TET2, inducing the MMP-9 promoter demethylation, which lead to MMP-9 upregulation and impaired healing. [86]

LncRNA URIDS (upregulated in diabetic skin) was found highly expressed in the skin of diabetic rat model. Accordingly, silencing lncRNA URIDS at the wound site, with an adenovirus expressing lnc-URIDS shRNA, accelerated *in vivo* wound closure. The lncRNA URIDS regulated wound healing through interaction with Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1 (PLOD1), which results in decreased collagen deposition and delayed healing. [87]

Understanding the role of lncRNAs in DFU is still in its genesis, thus emphasizing the need of further investigation to determine their potential for clinical application in the future.

## > CIRCULAR RNAs IN DIABETIC WOUND HEALING

CircRNAs are a class of non-coding RNA molecules that are structurally connected end to end to form single-chain molecules with a covalently closed loop structure. [88, 89] Functionally, circRNAs can regulate the level of transcriptional and post-transcriptional parental genes by acting as miRNA sponges. [88] The circRNAs bind and sequester transcriptionally inhibitory miRNAs, introducing a new level of gene expression regulation. [90, 91] The circRNAs involved in diabetic wound healing are indicated in Table 2.

CircRNAs have been reported to play a role in wound healing and a growing body of evidence implicate circRNAs in chronic wound healing. [92-96] The circRNA hsa_circ_0084443 was found upregulated in DFU. The overexpression of hsa_circ_0084443 in human keratinocytes decreased migration, [93] most likely through the modulation of PI3K, epidermal growth factor receptor (EGFR)

and extracellular signal-regulated kinase (ERK) signaling pathways as shown by transcriptomic analysis.

Due to their high stability, both circRNAs and miRNAs have been considered as potential biomarkers for various disorders, including non-healing ulcers. The hsa_circ_0000907 and hsa_circ_0057362 were found in serum and serum-derived exosomes were screened for the early diagnosis of DFU. [35, 36]

The role of circRNAs as regulators of the wound healing process in DFU is not clear, and more relevant studies are needed to clarify the relationship between them in the future. The identification and functional characterization of circRNAs holds a potential for novel miRNA inhibitory treatments for DFU associated with an abnormal miRNA expression.

## > CONCLUSIONS

Diabetes is becoming more and more prevalent in the world. Patients with diabetes suffer from numerous complications and DFU is one of the most common and serious. Indeed, DFU can lead to amputation, or even death, if it is not properly treated. This imposes a huge financial and health burden on patients with DFU worldwide. Hence, there is an urgent need for the development of therapeutics to improve the challenging treatment of DFU. This work highlights the importance of epigenetic, particularly DNA methylation and demethylation modifications, and regulation of non-coding RNAs, in wound healing under diabetic conditions, and provides valuable insights into the development of therapeutics targeting diabetic wound healing.

In wound healing, epigenetic mechanisms play a key role as regulators of cellular responses through all phases of wound healing progression. Further characterization of these mechanisms in wounded skin at the different stages will allow a better understanding of the transition of acute wound into chronic non-healing wounds. The study of the changes in epigenetic mechanisms in patients affected with DFU will provide directions for identification of novel therapeutic targets.

Several questions are raised for the use of epigenetic mechanisms as therapeutic strategies for DFUs. The regulation of DNA methylation and demethylation is variable among different cells under diverse diabetic conditions, making it challenging to ascertain how the variations in DNA methylation, the level of hyper- or hypomethylation and the specific sites, impact diabetic wound healing. Besides, caution should be taken to the use of non-coding RNAs as therapeutic targets for impaired wound healing as the pathology is very complex

with the malfunction of multiple cells, often involving more than a single non-coding RNA.

Moreover, epigenetic mechanisms can also be considered for potential application as markers for diagnosis and prognosis of wound healing disorders. Especially, the high stability and abundance of circRNAs and miRNAs contribute to its potential use as predictive and diagnostic biomarkers with the potential to reduce risks associated with chronic diabetic ulcers.

Therefore, further studies are needed to better understand the epigenetic targets of diabetic wound healing, to develop more effective therapeutic strategies for DFU treatments and to identify novel diagnostic and therapeutic targets. <

## REFERENCES

1. Armstrong DG, Boulton AJM, Bus SA. Diabetic Foot Ulcers and Their Recurrence. N Engl J Med. 2017 Jun 15; 376(24): 2367-2375. doi: 10.1056/NEJMra1615439.

2. Lo ZJ, Surendra NK, Saxena A, Car J. Clinical and economic burden of diabetic foot ulcers: A 5-year longitudinal multi--ethnic cohort study from the tropics. Int Wound J. 2021 Jun; 18(3): 375-386. doi: 10.1111/iwj.13540.

3. Walsh JW, Hoffstad OJ, Sullivan MO, Margolis DJ. Association of diabetic foot ulcer and death in a population-based cohort from the United Kingdom. Diabet Med. 2016 Nov; 33(11): 1493-1498. doi: 10.1111/dme.13054.

4. Da Silva J, Leal EC, Carvalho E. Bioactive Antimicrobial Peptides as Therapeutic Agents for Infected Diabetic Foot Ulcers. Biomolecules. 2021 Dec 17; 11(12): 1894. doi: 10.3390/biom11121894.

5. Leal EC, Carvalho E. Heme Oxygenase-1 as Therapeutic Target for Diabetic Foot Ulcers. Int J Mol Sci. 2022 Oct 10; 23(19): 12043. doi: 10.3390/ijms231912043.

6. Zeybel M, Mann DA, Mann J. Epigenetic modifications as new targets for liver disease therapies. J Hepatol. 2013 Dec; 59(6): 1349-53. doi: 10.1016/j.jhep.2013.05.039. 2

7. Herter EK, Xu Landén N. Non-Coding RNAs: New Players in Skin Wound Healing. Adv Wound Care (New Rochelle). 2017 Mar 1; 6(3): 93-107. doi: 10.1089/wound.2016.0711.

8. Natarajan R. Epigenetic Mechanisms in Diabetic Vascular Complications and Metabolic Memory: The 2020 Edwin Bierman Award Lecture. Diabetes. 2021 Feb; 70(2): 328-337. doi: 10.2337/dbi20-0030.

9. Boyko EJ, Zelnick LR, Braffett BH, Pop-Busui R, Cowie CC, Lorenzi GM, et al. Risk of Foot Ulcer and Lower-Extremity Amputation Among Participants in the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Study. Diabetes Care. 2022 Feb 1; 45(2): 357-364. doi: 10.2337/dc21-1816.

10. Chen Z, Zhang Y. Role of Mammalian DNA Methyltransferases in Development. Annu Rev Biochem. 2020 Jun 20; 89: 135-158. doi: 10.1146/annurev-biochem-103019-102815.

11. Niehrs C, Calkhoven CF. Emerging Role of C/EBPβ and Epigenetic DNA Methylation in Ageing. Trends Genet. 2020 Feb; 36(2): 71-80. doi: 10.1016/j.tig.2019.11.005.

12. den Dekker A, Davis FM, Kunkel SL, Gallagher KA. Targeting epigenetic mechanisms in diabetic wound healing. Transl Res. 2019 Feb; 204: 39-50. doi: 10.1016/j.trsl.2018.10.001.

13. Lewis CJ, Stevenson A, Fear MW, Wood FM. A review of epigenetic regulation in wound healing: Implications for the future of wound care. Wound Repair Regen. 2020 Nov; 28(6): 710-718. doi: 10.1111/wrr.12838.

14. Pastar I, Marjanovic J, Stone RC, Chen V, Burgess JL, Mervis JS, Tomic-Canic M. Epigenetic regulation of cellular functions in wound healing. Exp Dermatol. 2021 Aug; 30(8): 1073-1089. doi: 10.1111/exd.14325.

15. Singh K, Rustagi Y, Abouhashem AS, Tabasum S, Verma P, Hernandez E, et al. Genome-wide DNA hypermethylation opposes healing in patients with chronic wounds by impairing epithelial-mesenchymal transition. J Clin Invest. 2022 Sep 1; 132(17): e157279. doi: 10.1172/JCI157279.

16. Daley JM, Brancato SK, Thomay AA, Reichner JS, Albina JE. The phenotype of murine wound macrophages. J Leukoc Biol. 2010 Jan; 87(1): 59-67. doi: 10.1189/jlb.0409236.

17. Barman PK, Koh TJ. Macrophage Dysregulation and Impaired Skin Wound Healing in Diabetes. Front Cell Dev Biol. 2020 Jun 26; 8: 528. doi: 10.3389/fcell.2020.00528.

18. Knipper JA, Ding X, Eming SA. Diabetes Impedes the Epigenetic Switch of Macrophages into Repair Mode. Immunity. 2019 Aug 20; 51(2): 199-201. doi: 10.1016/j.immuni.2019.07.009.

19. Yan J, Tie G, Wang S, Tutto A, DeMarco N, Khair L, et al. Diabetes impairs wound healing by Dnmt1-dependent dysregulation of hematopoietic stem cells differentiation towards macrophages. Nat Commun. 2018 Jan 2; 9(1): 33. doi: 10.1038/s41467-017-02425-z.

20. Davis FM, Tsoi LC, Wasikowski R, denDekker A, Joshi A, Wilke C, et al. Epigenetic regulation of the PGE2 pathway modulates macrophage phenotype in normal and pathologic wound repair. JCI Insight. 2020 Sep 3; 5(17): e138443. doi: 10.1172/jci.insight.138443.

21. Singh K, Agrawal NK, Gupta SK, Mohan G, Chaturvedi S, Singh K. Genetic and epigenetic alterations in Toll like receptor 2 and wound healing impairment in type 2 diabetes patients. J Diabetes Complications. 2015 Mar; 29(2): 222-9. doi: 10.1016/j.jdiacomp.2014.11.015.

22. Fadini GP, Menegazzo L, Rigato M, Scattolini V, Poncina N, Bruttocao A, et al. NETosis Delays Diabetic Wound Healing in Mice and Humans. Diabetes. 2016 Apr; 65(4): 1061-71. doi: 10.2337/db15-0863.

23. Yasuda H, Takishita Y, Morita A, Tsutsumi T, Tsuchiya M, Sato EF. DNA demethylation increases NETosis. Arch Biochem Biophys. 2020 Aug 15; 689: 108465. doi: 10.1016/j.abb.2020.108465

24. Cannella V, Piccione G, Altomare R, Marino A, Di Marco P, Russotto L, et al. Differentiation and characterization of rat adipose tissue mesenchymal stem cells into endothelial-like cells. Anat Histol Embryol. 2018 Feb; 47(1): 11-20. doi: 10.1111/ahe.12318.

25. Shang T, Li S, Zhang Y, Lu L, Cui L, Guo FF. Hypoxia promotes differentiation of adipose-derived stem cells into endothelial cells through demethylation of ephrinB2. Stem Cell Res Ther. 2019 May 20; 10(1): 133. doi: 10.1186/s13287-019-1233-x.

26. Lee HJ, Hong YJ, Kim M. Angiogenesis in Chronic Inflammatory Skin Disorders. Int J Mol Sci. 2021 Nov 7; 22(21): 12035. doi: 10.3390/ijms222112035.

27. Hayashi SI, Rakugi H, Morishita R. Insight into the Role of Angiopoietins in Ageing-Associated Diseases. Cells. 2020 Dec 8; 9(12): 2636. doi: 10.3390/cells9122636.

28. Zhou H, Chen T, Li Y, You J, Deng X, Chen N, et al. Glycation of Tie-2 Inhibits Angiopoietin-1 Signaling Activation and Angiopoietin-1-Induced Angiogenesis. Int J Mol Sci. 2022 Jun 27; 23(13): 7137. doi: 10.3390/ijms23137137.

29. Zhao J, Yang S, Shu B, Chen L, Yang R, Xu Y, et al. Transient High Glucose Causes Persistent Vascular Dysfunction and Delayed Wound Healing by the DNMT1-Mediated Ang-1/NF-κB Pathway. J Invest Dermatol. 2021 Jun; 141(6): 1573-1584. doi: 10.1016/j.jid.2020.10.023.

30. Bandara N, Gurusinghe S, Kong A, Mitchell G, Wang LX, Lim SY, Strappe P. Generation of a nitric oxide signaling pathway in mesenchymal stem cells promotes endothelial lineage commitment. J Cell Physiol. 2019 Nov; 234(11): 20392-20407. doi: 10.1002/jcp.28640.

31. Park LK, Maione AG, Smith A, Gerami-Naini B, Iyer LK, Mooney DJ, et al. Genome-wide DNA methylation analysis identifies a metabolic memory profile in patient-derived diabetic foot ulcer fibroblasts. Epigenetics. 2014 Oct; 9(10): 1339-49. doi: 10.4161/15592294.2014.967584.

32. Liang Y, Yang C, Lin Y, Parviz Y, Sun K, Wang W, et al. Matrix metalloproteinase 9 induces keratinocyte apoptosis through FasL/Fas pathway in diabetic wound. Apoptosis. 2019 Aug; 24(7-8): 542-551. doi: 10.1007/s10495-019-01536-w.

33. Zhang J, Yang C, Wang C, Liu D, Lao G, Liang Y, et al. AGE-induced keratinocyte MMP-9 expression is linked to TET2-mediated CpG demethylation. Wound Repair Regen. 2016 May; 24(3): 489-500. doi: 10.1111/wrr.12426.

34. Ling L, Ren M, Yang C, Lao G, Chen L, Luo H, et al. Role of site--specific DNA demethylation in TNFα-induced MMP9 expression in keratinocytes. J Mol Endocrinol. 2013 Apr 12; 50(3): 279-90. doi: 10.1530/JME-12-0172.

35. Lindley LE, Stojadinovic O, Pastar I, Tomic-Canic M. Biology and Biomarkers for Wound Healing. Plast Reconstr Surg. 2016 Sep; 138(3 Suppl): 18S-28S. doi: 10.1097/PRS.0000000000002682.

36. Pastar I, Wong LL, Egger AN, Tomic-Canic M. Descriptive vs mechanistic scientific approach to study wound healing and its inhibition: Is there a value of translational research involving human subjects? Exp Dermatol. 2018 May; 27(5): 551-562. doi: 10.1111/exd.13663.

37. Gebert LFR, MacRae IJ. Regulation of microRNA function in animals. Nat Rev Mol Cell Biol. 2019 Jan; 20(1): 21-37. doi: 10.1038/s41580-018-0045-7.

38. Macfarlane LA, Murphy PR. MicroRNA: Biogenesis, Function and Role in Cancer. Curr Genomics. 2010 Nov; 11(7): 537-61. doi: 10.2174/138920210793175895.

39. Ha M, Kim VN. Regulation of microRNA biogenesis. Nat Rev Mol Cell Biol. 2014 Aug; 15(8): 509-24. doi: 10.1038/nrm3838.

40. Lewis BP, Shih IH, Jones-Rhoades MW, Bartel DP, Burge CB. Prediction of mammalian microRNA targets. Cell. 2003 Dec 26; 115(7): 787-98. doi: 10.1016/s0092-8674(03)01018-3.

41. Banerjee J, Sen CK. microRNA and Wound Healing. Adv Exp Med Biol. 2015; 888: 291-305. doi: 10.1007/978-3-319-22671-2_15. Banerjee J, Sen CK. microRNA and Wound Healing. Adv Exp Med Biol. 2015;888:291-305.

42. Mulholland EJ, Dunne N, McCarthy HO. MicroRNA as Therapeutic Targets for Chronic Wound Healing. Mol Ther Nucleic Acids. 2017 Sep 15; 8: 46-55. doi: 10.1016/j.omtn.2017.06.003.

43. Petkovic M, Sørensen AE, Leal EC, Carvalho E, Dalgaard LT. Mechanistic Actions of microRNAs in Diabetic Wound Healing. Cells. 2020 Oct 2; 9(10): 2228. doi: 10.3390/cells9102228.

44. Boon RA. Circulating MicroRNAs Link Inflammation to Impaired Wound Healing in Diabetes. Arterioscler Thromb Vasc Biol. 2015 Jun; 35(6): 1296-7. doi: 10.1161/ATVBAHA.115.305670.

45. Ban E, Jeong S, Park M, Kwon H, Park J, Song EJ, Kim A. Accelerated wound healing in diabetic mice by miRNA-497 and its anti-inflammatory activity. Biomed Pharmacother. 2020 Jan; 121: 109613. doi: 10.1016/j.biopha.2019.109613.

46. Xu J, Wu W, Zhang L, Dorset-Martin W, Morris MW, Mitchell ME, Liechty KW. The role of microRNA-146a in the pathogenesis of the diabetic wound-healing impairment: correction with mesenchymal stem cell treatment. Diabetes. 2012 Nov; 61(11): 2906-12. doi: 10.2337/db12-0145.

47. Li H, Han X, Zuo K, Li L, Liu J, Yuan X, et al. miR-23b promotes cutaneous wound healing through inhibition of the inflammatory responses by targeting ASK1. Acta Biochim Biophys Sin (Shanghai). 2018 Nov 1; 50(11): 1104-1113. doi: 10.1093/abbs/gmy109.

48. Mendell JT. miRiad roles for the miR-17-92 cluster in development and disease. Cell. 2008 Apr 18; 133(2): 217-22. doi: 10.1016/j.cell.2008.04.001.

49. Olive V, Li Q, He L. mir-17-92: a polycistronic oncomir with pleiotropic functions. Immunol Rev. 2013 May; 253(1): 158-66. doi: 10.1111/imr.12054.

50. Li D, Peng H, Qu L, Sommar P, Wang A, Chu T, et al. miR-19a/b and miR-20a Promote Wound Healing by Regulating the Inflammatory Response of Keratinocytes. J Invest Dermatol. 2021 Mar; 141(3): 659-671. doi: 10.1016/j.jid.2020.06.037.

51. Li X, Li D, Wang A, Chu T, Lohcharoenkal W, Zheng X, et al. MicroRNA-132 with Therapeutic Potential in Chronic Wounds. J Invest Dermatol. 2017 Dec; 137(12): 2630-2638. doi: 10.1016/j.jid.2017.08.003.

52. Ramirez HA, Pastar I, Jozic I, Stojadinovic O, Stone RC, Ojeh N, et al. Staphylococcus aureus Triggers Induction of miR-15B-5P to Diminish DNA Repair and Deregulate Inflammatory Response in Diabetic Foot Ulcers. J Invest Dermatol. 2018 May; 138(5): 1187-1196. doi: 10.1016/j.jid.2017.11.038.

53. Umehara T, Mori R, Mace KA, Murase T, Abe Y, Yamamoto T, Ikematsu K. Identification of Specific miRNAs in Neutrophils of Type 2 Diabetic Mice: Overexpression of miRNA-129-2-3p Accelerates Diabetic Wound Healing. Diabetes. 2019 Mar; 68(3): 617-630. doi: 10.2337/db18-0313.

54. Moura J, Sørensen A, Leal EC, Svendsen R, Carvalho L, Willemoes RJ, et al. microRNA-155 inhibition restores Fibroblast Growth Factor 7 expression in diabetic skin and decreases wound inflammation. Sci Rep. 2019 Apr 9; 9(1): 5836. doi: 10.1038/s41598-019-42309-4.

55. Dangwal S, Stratmann B, Bang C, Lorenzen JM, Kumarswamy R, Fiedler J, et al. Impairment of Wound Healing in Patients With Type 2 Diabetes Mellitus Influences Circulating MicroRNA Patterns via Inflammatory Cytokines. Arterioscler Thromb Vasc Biol. 2015 Jun; 35(6): 1480-8. doi: 10.1161/ATVBAHA.114.305048.

56. Da Silva J, Santos D, Vilaça M, Carvalho A, Carvalho R, Jesus Dantas M, et al. Impact of Psychological Distress on Physiological Indicators of Healing Prognosis in Patients with Chronic Diabetic Foot Ulcers: A Longitudinal Study. Adv Wound Care (New Rochelle). 2023 Nov 1. doi: 10.1089/wound.2023.0043.

57. Pereira MG, Vilaça M, Braga D, Madureira A, Da Silva J, Santos D, Carvalho E. Healing profiles in patients with a chronic diabetic foot ulcer: An exploratory study with machine learning. Wound Repair Regen. 2023 Nov-Dec; 31(6): 793-803. doi: 10.1111/wrr.13141.

58. Ozdemir D, Feinberg MW. MicroRNAs in diabetic wound healing: Pathophysiology and therapeutic opportunities. Trends Cardiovasc Med. 2019 Apr; 29(3): 131-137. doi: 10.1016/j.tcm.2018.08.002.

59. Pizzino G, Irrera N, Galfo F, Pallio G, Mannino F, D'amore A, et al. Effects of the antagomiRs 15b and 200b on the altered healing pattern of diabetic mice. Br J Pharmacol. 2018 Feb; 175(4): 644-655. doi: 10.1111/bph.14113.

60. Amin KN, Umapathy D, Anandharaj A, Ravichandran J, Sasikumar CS, Chandra SKR, et al. miR-23c regulates wound healing by targeting stromal cell-derived factor-1α (SDF-1α/CXCL12) among patients with diabetic foot ulcer. Microvasc Res. 2020 Jan; 127: 103924. doi: 10.1016/j.mvr.2019.103924.

61. Icli B, Nabzdyk CS, Lujan-Hernandez J, Cahill M, Auster ME, Wara AK, et al. Regulation of impaired angiogenesis in diabetic dermal wound healing by microRNA-26a. J Mol Cell Cardiol. 2016 Feb; 91: 151-9. doi: 10.1016/j.yjmcc.2016.01.007.

62. Wang JM, Tao J, Chen DD, Cai JJ, Irani K, Wang Q, et al. MicroRNA miR-27b rescues bone marrow-derived angiogenic cell function and accelerates wound healing in type 2 diabetes mellitus. Arterioscler Thromb Vasc Biol. 2014 Jan; 34(1): 99-109. doi: 10.1161/ATVBAHA.113.302104.

63. Huang C, Luo W, Wang Q, Ye Y, Fan J, Lin L, et al. Human mesenchymal stem cells promote ischemic repairment and angiogenesis of diabetic foot through exosome miRNA-21-5p. Stem Cell Res. 2021 Apr; 52: 102235. doi: 10.1016/j.scr.2021.102235.

64. Su M, Wang W, Liu F, Li H. Recent Progress on the Discovery of NLRP3 Inhibitors and their Therapeutic Potential. Curr Med Chem. 2021; 28(3): 569-582. doi: 10.2174/0929867327666200123093544.

65. Liang L, Stone RC, Stojadinovic O, Ramirez H, Pastar I, Maione AG, et al. Integrative analysis of miRNA and mRNA paired expression profiling of primary fibroblast derived from diabetic foot ulcers reveals multiple impaired cellular functions. Wound Repair Regen. 2016 Nov; 24(6): 943-953. doi: 10.1111/wrr.12470.

66. Wu D, Kang L, Tian J, Wu Y, Liu J, Li Z, et al. Exosomes Derived from Bone Mesenchymal Stem Cells with the Stimulation of Fe3O4 Nanoparticles and Static Magnetic Field Enhance Wound Healing Through Upregulated miR-21-5p. Int J Nano-medicine. 2020 Oct 19; 15: 7979-7993. doi: 10.2147/IJN.S275650.

67. Dallas A, Trotsyuk A, Ilves H, Bonham CA, Rodrigues M, Engel K, et al. Acceleration of Diabetic Wound Healing with PHD2- and miR-210-Targeting Oligonucleotides. Tissue Eng Part A. 2019 Jan; 25(1-2): 44-54. doi: 10.1089/ten.TEA.2017.0484.

68. Narayanan S, Eliasson Angelstig S, Xu C, Grünler J, Zhao A, et al. HypoxamiR-210 accelerates wound healing in diabetic mice by improving cellular metabolism. Commun Biol. 2020 Dec 14; 3(1): 768. doi: 10.1038/s42003-020-01495-y.

69. Wang W, Yang C, Wang XY, Zhou LY, Lao GJ, Liu D, et al. MicroRNA-129 and -335 Promote Diabetic Wound Healing by Inhibiting Sp1-Mediated MMP-9 Expression. Diabetes. 2018 Aug; 67(8): 1627-1638. doi: 10.2337/db17-1238.

70. Fang Y, Fullwood MJ. Roles, Functions, and Mechanisms of Long Non-coding RNAs in Cancer. Genomics Proteomics Bioinformatics. 2016 Feb; 14(1): 42-54. doi: 10.1016/j.gpb.2015.09.006.

71. Wilusz JE, Sunwoo H, Spector DL. Long noncoding RNAs: functional surprises from the RNA world. Genes Dev. 2009 Jul 1; 23(13): 1494-504. doi: 10.1101/gad.1800909.

72. Wilusz JE, Sunwoo H, Spector DL. Long noncoding RNAs: functional surprises from the RNA world. Genes Dev. 2009 Jul 1; 23(13): 1494-504. doi: 10.1101/gad.1800909.

73. Yao RW, Wang Y, Chen LL. Cellular functions of long noncoding RNAs. Nat Cell Biol. 2019 May; 21(5): 542-551. doi: 10.1038/s41556-019-0311-8.

74. Batista PJ, Chang HY. Long noncoding RNAs: cellular address codes in development and disease. Cell. 2013 Mar 14; 152(6): 1298-307. doi: 10.1016/j.cell.2013.02.012.

75. Beermann J, Piccoli MT, Viereck J, Thum T. Non-coding RNAs in Development and Disease: Background, Mechanisms, and Therapeutic Approaches. Physiol Rev. 2016 Oct; 96(4): 1297-325. doi: 10.1152/physrev.00041.2015.

76. Schmitz SU, Grote P, Herrmann BG. Mechanisms of long noncoding RNA function in development and disease. Cell Mol Life Sci. 2016 Jul; 73(13): 2491-509. doi: 10.1007/s00018-016-2174-5.

77. Wan DC, Wang KC. Long noncoding RNA: significance and potential in skin biology. Cold Spring Harb Perspect Med. 2014 May 1; 4(5): a015404. doi: 10.1101/cshperspect.a015404.

78. He X, Ou C, Xiao Y, Han Q, Li H, Zhou S. LncRNAs: key players and novel insights into diabetes mellitus. Oncotarget. 2017 Aug 4; 8(41): 71325-71341. doi: 10.18632/oncotarget.19921.

79. Hu J, Zhang L, Liechty C, Zgheib C, Hodges MM, Liechty KW, Xu J. Long Noncoding RNA GAS5 Regulates Macrophage Polarization and Diabetic Wound Healing. J Invest Dermatol. 2020 Aug; 140(8): 1629-1638. doi: 10.1016/j.jid.2019.12.030.

80. Zgheib C, Hodges MM, Hu J, Liechty KW, Xu J. Long non-coding RNA Lethe regulates hyperglycemia-induced reactive oxygen species production in macrophages. PLoS One. 2017 May 11; 12(5): e0177453. doi: 10.1371/journal.pone.0177453.

81. Li B, Luan S, Chen J, Zhou Y, Wang T, Li Z, et al. The MSC-Deri-

ved Exosomal lncRNA H19 Promotes Wound Healing in Diabetic Foot Ulcers by Upregulating PTEN via MicroRNA-152-3p. Mol Ther Nucleic Acids. 2020 Mar 6; 19: 814-826. doi: 10.1016/j.omtn.2019.11.034.

82. Li B, Zhou Y, Chen J, Wang T, Li Z, Fu Y, et al. Long noncoding RNA H19 acts as a miR-29b sponge to promote wound healing in diabetic foot ulcer. FASEB J. 2021 Jan; 35(1): e20526. doi: 10.1096/fj.201900076RRRRR.

83. Li D, Kular L, Vij M, Herter EK, Li X, Wang A, et al. Human skin long noncoding RNA WAKMAR1 regulates wound healing by enhancing keratinocyte migration. Proc Natl Acad Sci U S A. 2019 May 7; 116(19): 9443-9452. doi: 10.1073/pnas.1814097116.

84. Herter EK, Li D, Toma MA, Vij M, Li X, Visscher D, et al. WAK-MAR2, a Long Noncoding RNA Downregulated in Human Chronic Wounds, Modulates Keratinocyte Motility and Production of Inflammatory Chemokines. J Invest Dermatol. 2019 Jun; 139(6): 1373-1384. doi: 10.1016/j.jid.2018.11.033.

85. Jayasuriya R, Dhamodharan U, Karan AN, Anandharaj A, Rajesh K, Ramkumar KM. Role of Nrf2 in MALAT1/ HIF-1α loop on the regulation of angiogenesis in diabetic foot ulcer. Free Radic Biol Med. 2020 Aug 20; 156: 168-175. doi: 10.1016/j.freeradbiomed.2020.05.018.

86. Zhou L, Ren M, Zeng T, Wang W, Wang X, Hu M, et al. TET2--interacting long noncoding RNA promotes active DNA demethylation of the MMP-9 promoter in diabetic wound healing. Cell Death Dis. 2019 Oct 25; 10(11): 813. doi: 10.1038/s41419-019-2047-6.

87. Hu M, Wu Y, Yang C, Wang X, Wang W, Zhou L, et al. Novel Long Noncoding RNA lnc-URIDS Delays Diabetic Wound Healing by Targeting Plod1. Diabetes. 2020 Oct; 69(10): 2144-2156. doi: 10.2337/db20-0147.

88. Kristensen LS, Andersen MS, Stagsted LVW, Ebbesen KK, Hansen TB, Kjems J. The biogenesis, biology and characterization of circular RNAs. Nat Rev Genet. 2019 Nov; 20(11): 675-691. doi: 10.1038/s41576-019-0158-7.

89. Zhang Z, Yang T, Xiao J. Circular RNAs: Promising Biomarkers for Human Diseases. EBioMedicine. 2018 Aug; 34: 267-274. doi: 10.1016/j.ebiom.2018.07.036.

90. Hansen TB, Jensen TI, Clausen BH, Bramsen JB, Finsen B, Damgaard CK, Kjems J. Natural RNA circles function as efficient microRNA sponges. Nature. 2013 Mar 21; 495(7441): 384-8. doi: 10.1038/nature11993.

91. Memczak S, Jens M, Elefsinioti A, Torti F, Krueger J, Rybak A, et al. Circular RNAs are a large class of animal RNAs with regulatory potency. Nature. 2013 Mar 21; 495(7441): 333-8. doi: 10.1038/nature11928.

92. Li M, Wang J, Liu D, Huang H. High-throughput sequencing reveals differentially expressed lncRNAs and circRNAs, and their associated functional network, in human hypertrophic scars. Mol Med Rep. 2018 Dec; 18(6): 5669-5682. doi: 10.3892/mmr.2018.9557.

93. Wang A, Toma MA, Ma J, Li D, Vij M, Chu T, et al. Circular RNA hsa_circ_0084443 Is Upregulated in Diabetic Foot Ulcer and Modulates Keratinocyte Migration and Proliferation. Adv Wound Care (New Rochelle). 2020 Apr 1; 9(4): 145-160. doi: 10.1089/wound.2019.0956.

94. Wang J, Wu H, Xiao Z, Dong X. Expression Profiles of lncRNAs and circRNAs in Keloid. Plast Reconstr Surg Glob Open. 2019 Jun 20; 7(6): e2265. doi: 10.1097/GOX.0000000000002265.

95. Wu X, Xiao Y, Ma J, Wang A. Circular RNA: A novel potential biomarker for skin diseases. Pharmacol Res. 2020 Aug; 158: 104841. doi: 10.1016/j.phrs.2020.104841.

96. Yang ZG, Awan FM, Du WW, Zeng Y, Lyu J, Wu D, et al. The Circular RNA Interacts with STAT3, Increasing Its Nuclear Translocation and Wound Repair by Modulating Dnmt3a and miR-17 Function. Mol Ther. 2017 Sep 6; 25(9): 2062-2074. doi: 10.1016/j.ymthe.2017.05.022.