MicroRNA as Therapeutic Targets for Chronic Wound Healing

Mulholland, E., Dunne, N., & McCarthy, H. (2017). MicroRNA as Therapeutic Targets for Chronic Wound Healing. Molecular Therapy: Nucleic Acids, 8, 46-55. DOI: 10.1016/j.omtn.2017.06.003

Published in:
Molecular Therapy: Nucleic Acids

Document Version:
Publisher's PDF, also known as Version of record

Queen's University Belfast - Research Portal:
Link to publication record in Queen's University Belfast Research Portal

Publisher rights
Copyright 2017 the authors. This is an open access article published under a Creative Commons Attribution-NonCommercial-NoDerivs License (https://creativecommons.org/licenses/by-nc-nd/4.0/), which permits distribution and reproduction for non-commercial purposes, provided the author and source are cited.

General rights
Copyright for the publications made accessible via the Queen's University Belfast Research Portal is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The Research Portal is Queen's institutional repository that provides access to Queen's research output. Every effort has been made to ensure that content in the Research Portal does not infringe any person's rights, or applicable UK laws. If you discover content in the Research Portal that you believe breaches copyright or violates any law, please contact openaccess@qub.ac.uk.
MicroRNA as Therapeutic Targets for Chronic Wound Healing

Eoghan J. Mulholland,¹ Nicholas Dunne¹,²,³,⁴ and Helen O. McCarthy¹

¹School of Pharmacy, Queen’s University of Belfast, 97 Lisburn Road, Belfast BT9 7BL, UK; ²Centre for Medical Engineering Research, School of Mechanical and Manufacturing Engineering, Dublin City University, Stokes Building, Collins Avenue, Dublin 9, Ireland; ³Trinity Centre for Bioengineering, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin 2, Ireland; ⁴Department of Mechanical and Manufacturing Engineering, School of Engineering, Trinity College Dublin, Dublin 2, Ireland

Introduction to Wound Healing

Wound healing is a highly complex biological process composed of three overlapping phases: inflammation, proliferation, and remodeling. Impairments at any one or more of these stages can lead to compromised healing. MicroRNAs (miRs) are non-coding RNAs that act as post-transcriptional regulators of multiple proteins and associated pathways. Thus, identification of the appropriate miR involved in the different phases of wound healing could reveal an effective third-generation genetic therapy in chronic wound care. Several miRs have been shown to be upregulated or downregulated during the wound healing process. This article examines the biological processes involved in wound healing, the miR involved at each stage, and how expression levels are modulated in the chronic wound environment. Key miRs are highlighted as possible therapeutic targets, either through underexpression or overexpression, and the healing benefits are interrogated. These are prime miR candidates that could be considered as a gene therapy option for patients suffering from chronic wounds. The success of miR as a gene therapy, however, is reliant on the development of an appropriate delivery system that must be designed to overcome both extracellular and intracellular barriers.

Chronic Wounds

Chronic wounds are the result of multifactorial components within the wound healing process becoming compromised. They can be defined as a wound that is not continuously progressing toward healing or not healing in a methodical and timely fashion. Pathologically, one of the clearest indicators of a chronic wound is their failure to re-epithelialize. The aging population are among those most burdened with chronic wounds, and with lower keratinocyte cell turnover, this is a factor affecting their ability to re-epithelialize. In general, chronic wounds exhibit reduced mitogenic activity compared to acute wounds, which has been demonstrated not only in keratinocytes but also in skin fibroblasts. It has been shown that fibroblast cells isolated from venous leg ulcers have reduced cell migration properties in comparison to cells isolated from regular skin.

Overall, factors affecting healing ability are categorized as either local and/or systemic influences. Local refers to causes that directly affect the characteristics of the wound site itself and systemic factors related to the general overall health or disease state of the individual, which affects healing ability. As such, the following sections detail diabetes and infection as examples of a systemic and local factor, respectively, which can influence a wound to become chronically impaired. Differences in microRNA (miR) expression will also be explored within the sections miR and Wound Healing to miR Expression in Chronic Wounds.
Diabetes

Diabetes is a systemic factor that can be linked to wound healing and affects approximately 3.2 million people in the UK, which accounts for 6% of the population. This population is at risk of developing chronic non-healing diabetic foot ulcers. The increase in serum glucose has a significant influence on wound healing due to destructive effects showcased by hyperglycemia on the physiology of the affected cells. For example, sorbitol, a by-product of glucose metabolism, can accumulate within tissues, resulting in renal and vascular complications. Hypoxic regions are prevalent in diabetic wounds and are characterized by an absence of endothelial cells, resulting in reduced angiogenesis and elongation of the inflammatory phase. Diabetic mice models have shown decreased restoration of vasculature in diabetic wounds, with hindered endothelial progenitor cell mobilization and homing and reduced levels of VEGF, compared to a non-diabetic controls in vivo.

Non-enzymatic glycosylation associated with hyperglycemia also inhibits the function of structural and enzymatic peptides. Diabetic animal models of wound healing show decreased rates of granulation. Likewise, in humans, the findings were comparable, showing slow wound maturation and a lower count of fibroblast cells compared to non-diabetic wound sites.

Several cellular functions are affected in diabetic wounds, such as defective T cell immunity, leukocyte chemotaxis, phagocytosis, bactericidal capacity, and dysfunctions of fibroblasts and epidermal cells. Taken together, such defects contribute to inadequate clearance of infection and delayed healing.

Obesity is intrinsically linked to diabetes (type 2) and impaired wound healing. By 2050 in the UK, obesity is projected to affect 60% of adult men, 50% of adult women, and 25% of children. Obese patients have been reported to experience wound complications more frequently in relation to post-surgery healing, such as higher rates of infection. This has been linked to ischemia and hypo-perfusion in the subcutaneous adipose tissue. Skin folds also harbor microorganisms that thrive in moist areas and contribute to infection and tissue breakdown.

Infection is an example of a local factor that is linked to impaired wound healing. Virtually all open wounds experience colonization of microorganisms, which typically has no clinical consequence because no infection is evident. However, infection within a wound site can influence the healing process by prolonging the inflammatory phase. This is due to incomplete clearance of the wound site. Therefore, there is an elevated level of cytokines (e.g., interleukin-1 [IL-1] and tumor necrosis factor alpha [TNF-α]), which are pro-inflammatory. If the bacterial count within the wound is more than 105 colony-forming units per gram (CFUs/g) of tissue, the wound will not heal, irrespective of the treatment regime (e.g., skin graft placement and primary sutures). Furthermore, no healing will occur if the β-hemolytic strain of *Streptococcus* is present at the wound site, even at less than 105 CFUs/g.

Bacterial biofilms are a huge hurdle to overcome in the healing process for chronic wounds. A biofilm is an intricate community of microorganisms, characterized by massive cell densities that are accompanied by an extracellular polymer matrix, made mainly of polysaccharides and proteins. This matrix is multifunctional, acting both as a physical protector from biological and pharmaceutical antimicrobials. Further to this, it facilitates the adhesion of bacteria to surfaces, particularly foreign bodies.

Current Treatments

Gauze has been historically popular for wound dressings. However, the use of gauze promotes desiccation of the wound base, which is not advantageous for efficient healing. Furthermore, dry gauze often adheres to the surface of the wound, which can be painful for the patient upon removal. Such gauze dressings are susceptible to complete saturation of wound fluid and so can be ineffective in the protection against bacterial invasion. Films are alternatives to gauze dressings and can be utilized as multifunctional adhesive dressings, as in they can be used as primary dressings directly on the wound or alternatively as a secondary dressing to secure other primary dressings to the wound. Film membranes are thin and semi-permeable, enabling the exchange of oxygen and water vapor to the wound in addition to preventing liquid and microorganism contaminants.

Topical antiseptic and antibiotic treatments can be employed to reduce the bioburden of the wound. Limitations associated with topical antiseptics and antibiotics include toxicity to human tissue that can lead to prolonged inflammation and microbial resistance, in addition to the necessity for many reapplications to achieve the desired effects. Acetic acid is an example of a topical treatment typically administered at concentrations of between 0.25% and 1.0% (v/v). It is effective against most gram-positive and gram-negative organisms. Acetic acid is an inexpensive product; however, it can cause cytotoxicity in vitro and has limited activity against biofilms. The latter is a particular problem associated with chronic wounds.

The management and treatment of chronic wounds is influenced by many multifactorial elements; however, the current modes of treatments and dressing are largely inadequate for chronic wounds. Therefore, miR replacement or inhibitory therapy is an alternative mode of treatment because miR has the potential to affect an array of downstream gene targets.

miR

miRs are instrumental in the regulation of gene expression through the promotion of mRNA degradation and inhibition of mRNA translation. Intronic miRs are encoded within a gene transcript precursor, sharing the same promoter with the encoded gene transcripts. Unidentified promoters transcribe intergenic miR, located in the non-coding regions. Finally, polycistronic miRs derived from primary transcripts contain a diverse range of hairpins, giving rise to different miRs.

The process of miR biogenesis (Figure 1) is a highly complex process. In mammalian cells, miRs are transcribed by RNA polymerase II.
Primary miR (pri-miR) is cleaved by the RNase III intra-nuclear enzyme (i.e., Drosha) into many miR precursors (pre-miRs). Drosha and DGCR8/Pasha establish the microprocessor complex, which cuts the pri-miR at the ssRNA/dsRNA junction found at the base of the pri-miR hairpins and results in pre-miR production. The resulting pre-miRs are ~65 nt in length, with characteristic stem-loop hairpin secondary structures. The pre-miR is bound by Exportin 5 in the presence of the co-factor Ran-guanine triphosphatase (Ran-GTP) and is transported to the cell cytoplasm. Once the pre-miR has moved to the cytoplasm, a second RNase III enzyme (i.e., Dicer) processes the pre-miR with the help of several co-factors (e.g., double-stranded RNA-activated protein kinase [PACT] and TAR RNA-binding protein [TRBP]). Processing by Dicer leads to a mature miR strand and an opposing strand that degrades. Thus, the miR is incorporated into the RNA-induced silencing complex (RISC).

miR Function

miR modulates gene expression through association of an Argonaute protein, which loads the miR into the RISC at the 3’ end. The incorporated miR guides the RISC to its specific mRNA target through base-pair complementarity, which results in the disruption of translation.

Depending on the grade of complementarity between the target mRNA and the miR, two different mechanisms of RISC-mediated gene regulation may ensue. In the case of almost complete complementarity, the RISC cleaves the target mRNA, preventing translation. In the case of imperfect complementarity, translation is suppressed by miR decapping and/or deadenylation (Figure 1).

miR and Wound Healing

Several studies have demonstrated that miR expression is upregulated or downregulated during the overlapping phases of wound healing. Table 1 details key miRs that have been found to be instrumental during the different phases of wound healing. Therefore, provided the optimal miR can be identified, new therapies could be developed.

miR and the Inflammatory Phase

Inflammatory response and resolve is paramount to successful and rapid wound healing. Due to the tight regulation of pro-inflammatory and anti-inflammatory signaling, disruption of miR biogenesis may result in an imbalance of these signals adversely affecting the healing cascade. Evolving studies demonstrate that miR-21, miR-146a/b, and miR-155 play key roles in regulating the inflammatory process. miR-146 and miR-155 are promoted by TNF-α and IL-1β, and miR-146 has been shown to silence interleukin-1 receptor-associated kinase 1 (IRAK) and cyclooxygenase-2 (COX2), whereas miR-155 silences Src homology 2 domain-containing inositol 5-phosphatase (SHIP1), suppressor of cytokine signaling 1 (SOCS1), and IL-12.

miR and the Proliferation Phase

Proliferation of keratinocyte cells is critical for re-epithelialization of the wound, ergo miR regulation is vital. For this process to be efficient and progress rapidly, an abundant oxygen and nutrient supply is required and angiogenesis is key. Keratinocytes have been shown to migrate faster upon silencing of SHIP2 and the enhanced activation of the serine-threonine protein kinase AKT signaling pathway. miR-205 has been shown to suppress SHIP2, a negative regulator of AKT pathway and enhance collagen expression by a reduction in the regulation of phosphor-cofilin, which increases cell motility. Another important miR is miR-21, which has been shown to be an inhibitor of phosphatase and tensin homolog PTEN. PTEN inhibits the AKT pathway, thereby activating many cell survival and proliferative pathways.

miR and the Remodeling Phase

An important aspect of this phase of wound healing is the deposition of collagen. It has been observed that miR-29a has a direct influence on the expression of collagen at a post-transitional level. Fetal skin from mamals can heal without scarring. However, during advanced gestation, the transition is to a scarring phenotype. miR-192, miR-29b, and miR-29c are highly induced in the later phase of this process. Transforming growth factor β (TGF-β) (anti-fibrotic) and proteins such as SMADs that are involved in the pathways for scar-free healing are targets of miR-29b/c. miR-192 also enhances collagen 1α-2 expression through the targeting of SMAD-interacting protein 1 (SIP1).
miR Expression in Chronic Wounds

It has also been observed that many miRs expressed during the normal wound healing process are dysregulated during chronic wound healing. A recent study investigating the expression of miRs in rodents induced with diabetes showed that 18 miRs were upregulated and 65 miRs were downregulated compared to control wound healing groups (i.e., non-diabetic rodents). Therefore, many different miRs could be used as a therapeutic in the healing of chronic wounds. On the basis of the amalgamation of these observations, the following sections explore several specific miRs and show how they could be exploited for advanced wound healing applications.

miR-21

Murine studies have demonstrated that miR-21 plays an important role in keratinocyte migration and re-epithelialization during wound healing. miR-21 has been found to be upregulated during wound healing, coincident with TGF-β temporal expression. Consistently, knockdown of endogenous miR-21 using a specific antagomir dramatically delayed re-epithelialization, possibly due to the reduced keratinocyte migration. Likewise, Yang et al. found that through the utilization of wound scratch healing assays, transfection with miR-21 significantly increased keratinocyte cell migration by 62% compared to scrambled controls. TIMP3 and TIAM1 are regulated by miR-21 in vitro and in vivo. TIMP3 inhibits the activation of the EDK pathway with downstream activation of ERK1/2, thus promoting proliferation. Therefore, it can be postulated that miR-21 promotes keratinocyte migration and boosts re-epithelialization during skin wound healing.

miR-21 has also been found to be a key regulator of vascular smooth muscle cell proliferation and apoptosis via BCL2 activation and PTEN suppression. It also regulates MMP-2 via the PTEN pathway as a result of myocardial infarction. Inhibition of the PTEN pathway also activates many downstream targets, such as mTOR, which regulates protein synthesis, glucose homeostasis, autophagy, and proliferation (Figure 2). This suggests that introduction of miR-21 within the diabetic chronic wound site could have potential as a therapeutic by increasing the extent of cell migration.

miR-424

In normal wound healing, miR-424 is upregulated in response to hypoxic conditions (Figure 3). It works through the stabilization of transcription factor HIF-α. In normoxic conditions, oxygen targets HIF for degradation by post-translational hydroxylation at specific prolyl residues domains (PHD) within these subunits. This increases the affinity for the ubiquitin ligases for proteolytic destruction by the ubiquitin/proteasome pathway. The oxygen-dependent hydroxylation process, however, is suppressed during hypoxia, leading to stabilization of HIF-1α and subsequent attachment to its constitutive partner HIF-1β to induce transactivation. Stabilization of HIF has been classically known to induce the transcription of coding genes (e.g., vascular endothelial growth factor [VEGF], erythropoietin, and nitric oxide synthase-2).

| miR Involved in the Normal Wound Healing Process |
|-----------------------------------------------|
| miR   | Targets |
|-------|---------|
| Pro-inflammatory |
| miR-155 | SOCS1, SHIP1, IL-12 |
| miR-140 | PDGF |
| miR-16 | COX2 |
| miR-105 | TLR2 |
| miR-21 | PDCD4, PTEN |
| miR-125b | TNF-α |
| miR-233 | Mecl |
| miR-203 | TNF-α, IL-24 |
| miR-146a,b | TRAF6, IRAK1, STAT1, TNF-α, COX2 |
| Anti-inflammatory |
| miR-21 | TIMP3, TIAM1 |
| miR-31 | EMP-1 |
| miR-155 | KGF, FGF-7 |
| miR-99 | IGF1R, mTOR, AKT1 |
| miR-198 | DIAPH1, PLAU, LAMC2 |
| miR-184 | AKT |
| miR-205 | SHIP2, Rho-ROCK1 |
| miR-203 | RAN, RAPH1 |
| miR-210 | E2F3, ISCU1, ISCU2 |
| miR-483-3p | MK2, MK167, YAP1 |
| miR-21-7p | TIMP1 |
| miR-17-92 | TSP-1, CTGF |
| miR-31 | FIH-1, Spred1 |
| miR-126 | Spred1, PIK2R2 |
| miR-130a | GAX, HOXA5 |
| miR-210 | EFNA3 |
| miR-296 | HGS |
| miR-378 | Fus-1, Sufa |
| miR-424 | CUL2 |
| miR-92a | Integrin-α5 |
| miR-17 | JAK1 |
| miR-15b | VEGF |
| miR-16 | VEGF |
| miR-20a | MKK3, VEGF |
| miR-20b | HIF-1α, VEGF |
| miR-221 | c-kit |
| miR-222 | c-kit |
| miR-320 | IGF-1 |
| miR-503 | CCNE1, edc25A |
| miR-29a | TAB-1, collagen I and II |
| miR-29b | SMADs, β-catenin |
| miR-29c | SMADs, β-catenin |
| miR192/215 | E-cadherin, SIP1 |

| miR-155 | SOCS1, SHIP1, IL-12 |
| miR-140 | PDGF |
| miR-16 | COX2 |
| miR-105 | TLR2 |
| miR-21 | PDCD4, PTEN |
| miR-125b | TNF-α |
| miR-233 | Mecl |
| miR-203 | TNF-α, IL-24 |
| miR-146a,b | TRAF6, IRAK1, STAT1, TNF-α, COX2 |

Table 1. miR Involved in the Normal Wound Healing Process

www.moleculartherapy.org
With regards to miR-424, this results in transactivation genes that govern angiogenesis and metabolic pathways. miR-424 cleaves to the CUL2 3′ UTR and inhibits CUL2 expression. A drop in CUL2 levels leads to the destabilization of VCBCR U3-ligase complex, which leads to the stabilization and nuclear translocation of HIF-1α in endothelial cells. In vitro wound scratch assays found that miR-424 significantly increased cell migration, with endothelial cells transfected with miR-424, decreasing the wound width by 40% compared to a 16% decrease in the control group. Furthermore, angiogenesis tubular formation assays with endothelial cells transfected with miR-424 also showed increased tube establishment. This miR (mu-miR-322), which is the rodent homolog of miR-424, has been demonstrated to downregulate in a diabetic rodent model. However, in rodent models with ischemia, it has been shown that this miR is significantly upregulated. If there are prolonged periods of hypoxia, the natural response of cells is the initiation of apoptosis. Angiogenesis is crucial for rapid wound healing because it provides nutrients and oxygen to the wound site. With angiogenesis being compromised in patients with diabetes, it is crucial that it is stimulated to progress healing. Furthermore, fibroblasts that are exposed to prolonged periods of hypoxia may not participate in the formation of extracellular matrix (ECM), which again causes delays in healing. miR-424 is, therefore, a promoter of angiogenesis, validated by in vitro and in vivo rodent models.

miR-31

Human wound healing studies have demonstrated that miR-31 upregulation occurs in keratinocytes at the wound edge during proliferation. It has also been shown that overexpression of miR-31 increases proliferation, leading to an increased rate in re-epithelialization. Given that a low rate of re-epithelialization is typical of chronic wounds, miR-31 may be able to aid in the rapid closure of the wound.

Both TGF-β1 and TGF-β2 have been found to be upregulated in wounds and promote keratinocyte migration. TGF-β1 and TGF-β2 receptor binding results in the activation of downstream SMAD proteins, which in turn induce the expression of TGF-β.
target genes. Epithelial membrane protein-1 (EMP-1) has been identified as a suppresser of keratinocyte cell proliferation and migration (Figure 4). miR-31 expression is regulated by TGF-β2 and is a direct target of EMP-1. Silencing of EMP-1 expression results in a decreased level of miR-31 in keratinocytes; this suggests that a high level of EMP-1 during inflammation may trigger miR-31 expression.\(^{49}\)

Another target of miR-31 is factor-inhibiting HIF-1 (FIH-1) (Figure 5), which is an asparaginyl B-hydroxylase enzyme. It has been shown that FIH-1 regulates HIF-1 and subsequently VEGF, thus having a direct effect on the rate of angiogenesis. Upregulation of miR-31 would therefore increase the levels of VEGF through the silencing of FIH-1.\(^{50}\)

Furthermore, it has been demonstrated that miR-31 is downregulated in wounds within the diabetic rodent model.\(^{42}\) Taken together, all the evidence indicates that delivery of miR-31 is a candidate therapy for the inflammatory and proliferative phases of wound healing.

miR-221 and miR-222
Infection is another key consideration in the wound healing process, and nitric oxide (NO) is a potent antibacterial agent. miR-221 and miR-222 have been found to reduce the expression of endothelial NO, which is essential for many cellular functions.\(^{51}\) Many studies have demonstrated that NO levels are lower in diabetic wounds.\(^{52,53}\) In vivo studies using eNOS knock-out mice have demonstrated delayed closure of wounds\(^{54}\) and a reduced rate of angiogenesis.\(^{55}\) Thus, the silencing of miR-221 and miR-222 could serve as a potential therapeutic to target infection in chronic wound healing.

Modes of Upregulating/Downregulating miR
For the introduction of miR into cells, many types of cargo could be utilized, for example, plasmid DNA coding for specific miRs to upregulate expression or antagonirs for silencing of expression.\(^{56,57}\) For this, numerous genetic delivery systems are currently utilized in research, with viral vectors being the most efficient.\(^{58}\) However, there are apprehensions surrounding viral vectors as genetic carriers due to mutagenesis, toxicity, and its limited capacity for genetic cargo.\(^{59}\) Examples of non-viral options include polymers of cationic nature, liposomes, and peptides, which exhibit the ability to not only package genetic cargo, but also deliver it to the nucleus of cells.\(^{60}\) Liposomes are composed of a membrane composed of lipids, in which nucleic acid can be encapsulated within. Liposomes can take three forms: anionic, neutral, and, cationic, with cationic being the most frequently

---

\(^{49}\) In vivo studies using eNOS knock-out mice have demonstrated delayed closure of wounds and a reduced rate of angiogenesis.

\(^{50}\) The silencing of miR-31 and miR-222 could serve as a potential therapeutic to target infection in chronic wound healing.

\(^{51}\) Examples of non-viral options include polymers of cationic nature, liposomes, and peptides, which exhibit the ability to not only package genetic cargo, but also deliver it to the nucleus of cells.

\(^{52}\) Liposomes are composed of a membrane composed of lipids, in which nucleic acid can be encapsulated within.
utilized for nucleic acid delivery due to its efficacy in interacting with cell membranes.61 There are many commercial options of cationic liposomes, such as Lipofectamine RNAi-MAX (Invitrogen)62 or SiPORT (Invitrogen).63 In vitro assays looking at the efficiency of liposomes at transfecting non-small-cell lung cancer cells with miR-29b found that there was a 5-fold increase in their expression compared to non-treated cells, validating their power as an miR delivery system.64 For wound healing, the ideal non-viral delivery vector must be non-immunogenic and non-toxic so as not to compromise the already delicate wound tissue.

Conclusions

Utilization of miR presents an attractive proposition for the development of therapeutic targets that could act on various pathways associated with chronically impaired wound healing. With varying levels of many miRs during the different phases of wound healing, there are several possible targets that could be employed.

Examples of prime miR candidates for wound healing include miR-21 and miR-31. miR-21, a target for the proliferation and inflammatory phase, could stop inflammation by targeting PDCD4 and promote proliferation and cell survival by activation of the mTOR pathway. miR-31 targets FIH-1; this increases the levels of VEGF intracellularly and protected micro-environment in addition to the efficacious delivery of the miR gene therapy. It can be rationalized, therefore, successful delivery of the therapeutic.

However, the rate-limiting factor in the implementation of miR therapy is a delivery system. This delivery system must be designed to overcome extracellular and intracellular barriers to ensure the successful delivery of the therapeutic cargo to the desired targets without evoking an immune response. Because it is of principal importance to maintain an optimum healing environment, it would be prudent to incorporate a gene therapy delivery system as part of a dressing instrument. Adopting such a system could confer multi-functionality with the dressing, ensuring a moist and protected micro-environment in addition to the efficacious delivery of the miR gene therapy. It can be rationalized, therefore, that miR holds the potential to become a momentous third-generation nucleic acid therapeutic for the treatment and management of chronic wounds, provided the optimal targeting and delivery system can be designed.

REFERENCES

1. Tobin, D.J. (2006). Biochemistry of human skin–our brain on the outside. Chem. Soc. Rev. 35, 52–67.
2. Kanitakis, J. (2002). Anatomy, histology and immunohistochemistry of normal human skin. Eur. J. Dermatol. 12, 390–399.
3. Templeton, N., ed. (2015). Gene and Cell Therapy (CRC Press).
4. Guttner, G.C., Werner, S., Barrandon, Y., and Longaker, M.T. (2008). Wound repair and regeneration. Nature 453, 314–321.
5. Gould, L., Abadir, P., Brem, H., Carter, M., Conner-Kerr, T., Davidson, J., DiPietro, L., Falanga, V., Fife, C., Gardner, S., et al. (2015). Chronic wound repair and healing in older adults: current status and future research. J. Am. Geriatr. Soc. 63, 427–438.
6. Guo, S., and DiPietro, L.A. (2010). Factors affecting wound healing. J. Dent. Res. 89, 219–229.
7. Werner, S., and Antuñes-Roca, M. (2016). Wound healing: an orchestrated process of cell cycle, adhesion, and signaling. In Encyclopedia of Cell Biology (Elsevier), pp. 216–222.
8. Orgill, D., and Blanco, C. (2009). Biomaterials for Treating Skin Loss (Elsevier).
9. Falanga, V. (2004). The chronic wound: impaired healing and solutions in the context of wound bed preparation. Blood Cells Mol. Dis. 32, 88–94.
10. Adair, H.M. (1977). Epidermal repair in chronic venous ulcers. Br. J. Surg. 64, 800–804.
11. Grove, G.L., and Kligman, A.M. (1983). Age-associated changes in human epidermal cell renewal. J. Gerontol. 38, 137–142.
12. Raffetto, J.D., Mendez, M.V., Marien, B.J., Byers, H.R., Phillips, T.L, Park, H.Y., and Menzoian, J.O. (2001). Changes in cellular motility and cytoskeletal actin in fibroblasts from patients with chronic venous insufficiency and in neonatal fibroblasts in the presence of chronic wound fluid. J. Vasc. Surg. 33, 1233–1241.
13. Diabetes UK. (2014). Diabetes: facts and stats. https://www.diabetes.org.uk/Documents/AboutUs/Statistics/Diabetes-key-stats-guidelines-April2014.pdf.
14. Broughton, G., 2nd, Janis, J.E., and Attinger, C.E. (2006). Wound healing: an overview. Plast. Reconstr. Surg. 117 (Suppl.), 1–S–32–S.
15. He, Z., and King, G.L. (2004). Microvascular complications of diabetes. Endocrinol Metab. Clin. North Am. 33, 215–238.
16. Brem, H., and Tomic-Canic, M. (2007). Cellular and molecular basis of wound healing in diabetics. J. Clin. Invest. 117, 1219–1222.
17. Gary Sibbald, R., and Woo, K.Y. (2008). The biology of chronic foot ulcers in persons with diabetes. Diabetes Metab. Res. Rev. 24 (Suppl 1), S25–S30.
18. Swanton, K. (2008). Healthy weight, healthy lives: a toolkit for developing local strategies. London Department of Health, http://webarchive.nationalarchives.gov.uk/20130107105354/http://www.dh.gov.uk/prod_consm_dh/groups/dh_digitalassets/documents/digitalasset/dh_088967.pdf.
19. Momeni, A., Heier, M., Bannasch, H., and Stark, G.B. (2009). Complications in ab- dominal liposuction: a risk factor analysis. J. Plast. Reconstr. Aesthet. Surg. 62, 1250–1254.
20. Fedly, E.R., Jones, D., Critchley, H.O.D., Phipps, R.P., Blieden, T.M., and Springer, T.A. (2001). Expression of stromal-derived factor-1 is decreased by IL-1 and TNF and in dermal wound healing. J. Immunol. 166, 5749–5754.
21. Robson, M.C. (1997). Wound infection. A failure of wound healing caused by an imbalance of bacteria. Surg. Clin. North Am. 77, 637–650.
22. Bowler, P.G., Duerden, B.L., and Armstrong, D.G. (2001). Wound microbiology and associated approaches to wound management. Clin. Microbiol. Rev. 14, 244–269.
23. Schierle, C.F., De la Garza, M., Mustoe, T.A., and Galano, R.D. (2009). Staphylococcal biofilms impair wound healing by delaying reepithelialization in a murine cutaneous wound model. Wound Repair Regen. 17, 354–359.
24. Fonder, M.A., Lazarus, G.S., Cohan, D.A., Aronson-Cook, B., Kohli, A.R., and Mamela, A.J. (2008). Treating the chronic wound: a practical approach to the care of nonhealing wounds and wound care dressings. J. Am. Acad. Dermatol. 58, 185–206.
25. Baranoski, S. (2008). Choosing a wound dressing, part 1. Nursing 38, 60–61.
26. Atyeh, B.S., Dibo, S.A., and Hayek, S.N. (2009). Wound cleansing, topical antisepsis and wound healing. Int. Wound J. 6, 420–430.
27. Bjarnsholt, T., Albede, M., Jensen, P.O., Nielsen, A.K., Johansen, H.K., Homoe, P., Haiby, N., Givskov, M., and Kirketerp-Moller, K. (2015). Antibiofilm properties of acetic acid. Adv. Wound Care (New Rochelle) 4, 363–372.
28. Lipsky, B.A., and Hoyer, C. (2009). Topical antimicrobial therapy for treating chronic wounds. Clin. Infect. Dis. 49, 1541–1549.
29. Bartel, D.P. (2004). MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116, 281–297.
30. Sand, M., Gambichler, T., Sand, D., Skrygan, M., Altmeyer, P., and Bechara, F.G. (2009). MicroRNAs and the skin: tiny players in the body’s largest organ. J. Dermatol. Sci. 53, 169–175.

31. Ha, M., and Kim, V.N. (2014). Regulation of microRNA biogenesis. Nat. Rev. Mol. Cell Biol. 15, 509–524.

32. Lee, Y., Ahn, C., Han, J., Choi, H., Kim, J., Yim, J., Lee, J., Provost, P., Radmark, O., Kim, S., et al. (2003). The nuclear RNPase III Drosha initiates microRNA processing. Nature 425, 415–419.

33. Kim, V.N., Han, J., and Siomi, M.C. (2009). Biogenesis of small RNAs in animals. Nat. Rev. Mol. Cell Biol. 10, 126–139.

34. Banerjee, J., Chan, Y.C., and Sen, C.K. (2011). MicroRNAs in skin and wound healing. Methods Mol. Biol. 43, 155–165.

35. Chendrimada, T.P., Finn, K.J., Ji, X., Baillat, D., Gregory, R.I., Liebhaber, S.A., Lee, Y., Ahn, C., Han, J., Choi, H., Kim, J., Yim, J., Lee, J., Provost, P., Radmark, O., Kim, S., et al. (2003). The nuclear RNPase III Drosha initiates microRNA processing. Nature 425, 415–419.

36. Roy, S., and Sen, C.K. (2011). MiRNA in innate immune responses: novel players in wound inflammation. Physiol. Genomics 43, 557–565.

37. Zhu, N., Zhang, D., Chen, S., Liu, X., Lin, L., Huang, X., Guo, Z., Liu, J., Wang, Y., Yuan, W., et al. (2011). Endothelial enriched microRNAs regulate angiogenesis II-induced endothelial inflammation and migration. Atherosclerosis 215, 286–293.

38. Yu, J., Ryan, D.G., Getzios, S., Oliveira-Fernandes, M., Fatima, A., and Lavker, R.M. (2008). MicroRNA-184 antagonizes microRNA-205 to maintain SHIP2 levels in epithelia. Proc. Natl. Acad. Sci. USA 105, 19300–19305.

39. Maurer, B., Stanczyk, J., Jüngel, A., Akhmetshina, A., Trenkmann, M., Brock, M., Kowal-Bielecka, O., Gay, R.E., Michel, B.A., Distler, J.H., et al. (2010). MicroRNA-29, a key regulator of collagen expression in systemic sclerosis. J. Clin. Invest. 120, 4141–4154.

40. Beanes, S.R., Dang, C., Soo, C., and Ting, K. (2003). Skin repair and scar formation: the central role of TGF-beta. Expert Rev. Mol. Med. 5, 1–22.

41. Kato, M., Zhang, J., Wang, M., Lanting, L., Yuan, H., Rossi, I.J., and Natarajan, R. (2007). MicroRNA-192 in diabetic kidney glomeruli and its function in TGF-beta-induced collagen expression via inhibition of E-box repressors. Proc. Natl. Acad. Sci. USA 104, 3432–3437.

42. Liu, Y.-F., Ding, M., Liu, D.-W., Liu, Y., Mao, Y.-G., and Peng, Y. (2015). MicroRNA profiling in cutaneous wounds of diabetic rats. Genet. Mol. Res. 14, 9614–9625.

43. Yang, X., Wang, J., Guo, S.L., Fan, K.J., Li, J., Wang, Y.L., Teng, Y., and Yang, X. (2013). MiRNA-1 antagonizes miRNA-145 to maintain SHIP2 levels in epithelia. Proc. Natl. Acad. Sci. USA 110, 10393–10398.

44. Banerjee, J., Shi, Z., Li, M., and Mi, J. (2014). Hypoxia-induced miR-24 decreases tumor sensitivity to chemotherapy by inhibiting apoptosis. Cell Death Dis. 5, e1301.

45. Li, D., Li, X., Wang, A., Meisgen, F., Pivarcsi, A., Sonkoly, E., Stähle, M., and Landén, N.X. (2015). MicroRNA-31 promotes skin wound healing by enhancing keratinocyte proliferation and migration. J. Invest. Dermatol. 135, 1676–1685.

46. Mahon, P.C., Hirota, K., and Semenza, G.L. (2001). FIH-1: a novel protein that interacts with HIF-1 alpha and VHL to mediate repression of HIF-1 transcriptional activity. Genes Dev. 15, 2675–2686.

47. Zhang, D., Shi, Z., Li, M., and Mi, J. (2014). Hypoxia-induced miR-24 decreases tumor sensitivity to chemotherapy by inhibiting apoptosis. Cell Death Dis. 5, e1301.

48. Suárez, Y., Fernández-Hernando, C., Poher, J.S., and Sessa, W.C. (2007). Dicer dependent microRNAs regulate gene expression and functions in human endothelial cells. Circ. Res. 100, 1164–1173.

49. Witte, M.B., Thornton, F.J., Tantriy, U., and Barbul, A. (2002). L-Arginine supplementation enhances diabetic wound healing involvement of the nitric oxide synthase and arginase pathways. Metabolism 51, 1269–1273.

50. Schäffer, M.R., Tantriy, U., Elron, P.A., Ahrendt, G.M., Thornton, F.J., and Barbul, A. (1997). Diabetes-impaired healing and reduced wound nitric oxide synthesis: a possible pathophysiologic correlation. Surgery 121, 513–519.

51. Xu, N., Brodin, P., Wei, T., Meisgen, F., Eidsmo, L., Nagy, N., Kemeny, L., Stähle, M., Sonkoly, E., and Pivarcsi, A. (2011). MiR-122b, a microRNA downregulated in psoriasis, modulates keratinocyte proliferation by targeting FGFR2. J. Invest. Dermatol. 131, 1521–1529.
Review

71. Sonkoly, E., Stähle, M., and Pivarcis, A. (2008). MicroRNAs: novel regulators in skin inflammation. Clin. Exp. Dermatol. 33, 312–315.

72. Johnnidis, J.B., Harris, M.H., Wheeler, R.T., Stehling-Sun, S., Lam, M.H., Kirak, O., Brummelkamp, T.R., Fleming, M.D., and Camargo, F.D. (2008). Regulation of progenitor cell proliferation and granulocyte function by microRNA-223. Nature 451, 1125–1129.

73. Primo, M.N., Bak, R.O., Schübler, B., and Mäkkelsen, I.G. (2012). Regulation of proinflammatory cytokines TNFα and IL24 by microRNA-203 in primary keratinocytes. Cytokine 60, 741–748.

74. Park, H., Huang, X., Lu, C., Cairo, M.S., and Zhou, X. (2015). MicroRNA-146a and microRNA-146b regulate human dendritic cell apoptosis and cytokine production by targeting TRAF6 and IRAK1 proteins. J. Biol. Chem. 290, 2831–2841.

75. Potter, N., Maurin, T., Chevalier, B., Puissegur, M.P., Lebrigand, K., Robbe-Sermesant, X., Bertero, T., Lino Cardenas, C.L., Couricot, E., Rios, G., et al. (2009). Identification of keratinocyte growth factor as a target of microRNA-155 in lung fibroblasts: implication in epithelial-mesenchymal interactions. PLoS One 4, e6718.

76. Jin, Y., Tymen, S.D., Chen, D., Fang, Z.J., Zhao, Y., Dragas, D., Dai, Y., Marucha, P.T., and Zhou, X. (2013). MicroRNA-99 family targets AKT/mTOR signaling pathway in dermal wounding. PLoS ONE 8, e64434.

77. Sundaram, G.M., Common, J.E.A., Gopal, F.E., Srikanta, S., Lakshman, K., Lunny, D.P., Lim, T.C., Tanavde, V., Lane, E.B., and Sampath, P. (2013). ‘See-saw’ expression of microRNA-198 and FSTL1 from a single transcript in wound healing. Nature 495, 103–106.

78. Yu, J., Peng, H., Ruan, Q., Fatima, A., Gettsios, S., and Lavker, R.M. (2010). MicroRNA-205 promotes keratinocyte migration via the lipid phosphate SHIP2. FASEB J. 24, 3950–3959.

79. Harris, T.A., Yamakuchi, M., Ferlito, M., Mendell, J.T., and Lowenstein, C.J. (2008). MicroRNA-126 regulates endothelial expression of vascular cell adhesion molecule 1. Proc. Natl. Acad. Sci. USA 105, 1314–1319.

80. Wang, W., Moneimeim, G., Sidani, M., Wyckoff, J., Chen, X., Makris, A., Goswami, S., Bresnick, A.R., and Condeelis, J.S. (2006). The activity status of coflin is directly related to invasion, intravasation, and metastasis of mammary tumors. J. Cell Biol. 173, 395–404.

81. Vitovich, G., Lena, A.M., Gianfaroni, F., Odorossi, T., Annichiarico-Petruzelli, M., Melino, G., and Candi, E. (2012). MicroRNA-203 contributes to skin re-epithelialization. Cell Death Dis. 3, e435.

82. Biswas, S., Roy, S., Banerjee, J., Hussain, S.R., Khanna, S., Meenakshisundaram, G., Kuppusamy, P., Friedman, A., and Sen, C.K. (2010). Hypoxia inducible microRNA-210 expression in myocardial microvascular endothelial cells and its relationship with insulin-like growth factor-1 in type 2 diabetic rats. Clin. Exp. Pharmacol. Physiol. 37, 14082–14087.

83. Bertero, T., Gastaldi, C., Bourget-Ponzio, I., Imbert, V., Loubat, A., Felli, N., Fontana, L., Pelosi, E., Botta, R., Bonci, D., Facchiano, F., Liuzzi, F., Lulli, V., Poliseno, L., Tuccoli, A., Mariani, L., Evangelista, M., Citti, L., Woods, K., Weisleder, R., Breakefield, X.O., and Krichevsky, A.M. (2008). miR-296 regulates growth factor receptor overexpression in angiogenic endothelial cells. Cancer Cell 14, 382–393.

84. Suárez, Y., Fernández-Hernando, C., Yu, J., Gerber, S.A., Harrison, K.D., Pober, J.S., Iruela-Arispe, M.L., Merkenschlager, M., and Sessa, W.C. (2008). Dicer-dependent regulation of microRNA-198 and FSTL1 from a single transcript in wound healing. Nature 451, 1125–1129.

85. Suárez, Y., Fernández-Hernando, C., Yu, J., Gerber, S.A., Harrison, K.D., Pober, J.S., Iruela-Arispe, M.L., Merkenschlager, M., and Sessa, W.C. (2008). Dicer-dependent endothelial microRNAs are necessary for postnatal angiogenesis. Proc. Natl. Acad. Sci. USA 105, 14082–14087.

86. Dewa, M., Homayouni, A., Yu, D., Murphy, D., Sevignani, C., Wentzel, E., Furth, E.E., Lee, W.M., Enders, G.H., Mendell, J.T., et al. (2006). Augmentation of tumor angiogenesis by a Myc-activated microRNA cluster. Nat. Genet. 38, 1060–1065.

87. Hu, J., Chen, C., Liu, Q., Liu, B., Song, C., Zhu, S., Wu, C., Liu, S., Yu, H., Yao, D., et al. (2015). The role of the miR-31/FH1 pathway in TGF-β-induced liver fibrosis. Clin. Sci. 129, 305–317.

88. Edmonds, M.D., Boyd, K.L., Moyo, T., Mitra, R., Dzusynski, R., Arrate, M.P., Chen, X., Zhao, Z., Blackwell, T.S., Andl, T., et al. (2016). MicroRNA-31 initiates lung tumorigenesis and promotes mutant KRAS-driven lung cancer. J. Clin. Invest. 126, 349–364.

89. Wang, S., Aurora, A.B., Johnson, B.A., Qi, X., McAnally, J., Hill, J.A., Richardson, J.A., Bassel-Duby, R., and Olson, E.N. (2008). The endothelial-spe-
106. Li, Z., Hassan, M.Q., Jafferji, M., Ageilan, R.I., Garzon, R., Croce, C.M., van Wijnen, A.J., Stein, J.L., Stein, G.S., and Lian, J.B. (2009). Biological functions of miR-29b contribute to positive regulation of osteoblast differentiation. J. Biol. Chem. 284, 15676–15684.

107. van Rooij, E., Sutherland, L.B., Thatcher, J.E., DiMaio, J.M., Naseem, R.H., Marshall, W.S., Hill, J.A., and Olson, E.N. (2008). Dysregulation of microRNAs after myocardial infarction reveals a role of miR-29 in cardiac fibrosis. Proc. Natl. Acad. Sci. USA 105, 13027–13032.

108. Wang, B., Herman-Edelstein, M., Koh, P., Burns, W., Jandeleit-Dahm, K., Watson, A., Saleem, M., Goodall, G.J., Twigg, S.M., Cooper, M.E., et al. (2010). E-cadherin expression is regulated by miR-192/215 by a mechanism that is independent of the profibrotic effects of transforming growth factor-beta. Diabetes 59, 1794–1802.