Dynamic changes of wound-related miRNAs after application of autologous platelet-rich gel in diabetic wounds

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To the Editor: Diabetic chronic cutaneous ulcers (DCUs) are among the most common and serious complications of diabetes mellitus. Diabetic foot ulcers are the most common type of DCU and are now considered a global epidemic.[1] The clinical prevention and treatment of DCUs are critical and have remained challenging for clinicians. With the exploration of topical treatment methods, autologous platelet-rich gel (APG) is regarded as an adjuvant therapy to promote wound healing, and it has been widely used for wound repair.[2] Some microRNAs that play a critical role in the whole wound healing process have been found in wound tissues, which we call wound-related miRNAs (wr-miRNAs). However, few studies have explored the dynamic changes of wr-miRNAs during APG treatment. Our study aimed to evaluate the efficacy of APG in treating diabetic wounds and explore the dynamic changes of wr-miRNAs during wound healing.

In this study, we established diabetic and non-diabetic cutaneous wound models in minipigs. The study was approved by the Animal Ethics Committee of West China Hospital, Sichuan University (No. IACUC 2020214A). Ten 2.5-month-old male Bama miniature pigs were used, of which eight were diabetic and two were non-diabetic. Diabetes was induced by a high-fat and high-carbohydrate diet combined with an intravenous injection of streptozotocin (STZ). One month after diagnosis of diabetes, we created 14 to 18 full-thickness defect wounds on each pig through surgery. After surgery, we filled the wounds with lidocaine-soaked gauze for analgesia and hemostasis. Subsequently, we used multiple methods to interfere with the normal wound healing process. For further details, please see [Supplementary Materials section, http://links.lww.com/CM9/B256].

The wounds of diabetic pigs were divided into the diabetic APG group ($n = 36$) and the diabetic control group ($n = 18$), and the wounds of non-diabetic pigs were divided into the non-diabetic control group ($n = 14$). Accordingly, the wounds in the two control groups received standard treatment, while the wounds in the diabetic APG group were treated with APG based on standard therapy. In all groups, dressings were changed every 3 days. Figure 1A illustrates the APG manufacturing process. Briefly, we first used the quadratic differential centrifugation method to extract platelet-rich plasma (PRP) from peripheral venous blood and then simultaneously pushed PRP and thrombin-calcium agent (TCA) into the wound to coagulate into a gelatinous substance, known as APG.

The wound area was measured once before treatment and every 3 days after treatment. Healing rate = [(initial area of wound–unhealed area)/initial area of wound] × 100%. Wound healing time (days) was recorded as the time when the wound was entirely covered by the epithelium. Wound tissues were collected using a biopsy device before treatment and 3, 6, 9, 12, and 15 days after treatment. A TakaraRNAiso Small RNA kit (Takara, Tokyo, Japan) was used to extract miRNAs, and a Bulge-Loop™ miRNA qRT-PCR Starter Kit (Ribobio, Guangzhou, China) was used for reverse transcription and amplification according to the manufacturer's instructions. miRNAs and the internal reference gene (U6) in each sample were reverse-transcribed separately. The reverse transcription primers were specific stem ring primers synthesized by Ruibo Biotechnology Co., Ltd. (Guangzhou, Guangdong, China). All reactions were performed using a CFX96 real-time system. D0 was used as a control to calculate the relative expression of miRNAs using $-\Delta\Delta ct$ and $2^{-\Delta\Delta ct}$.

All pigs injected with STZ met the diagnostic criteria for diabetes at 30 months of age, and all wounds remained unhealed for 8 weeks. We observed the wounds on the day of treatment and every 3 days after treatment. Generally, the wound healing tendency was similar, while the wound surfaces varied slightly between the three groups. The

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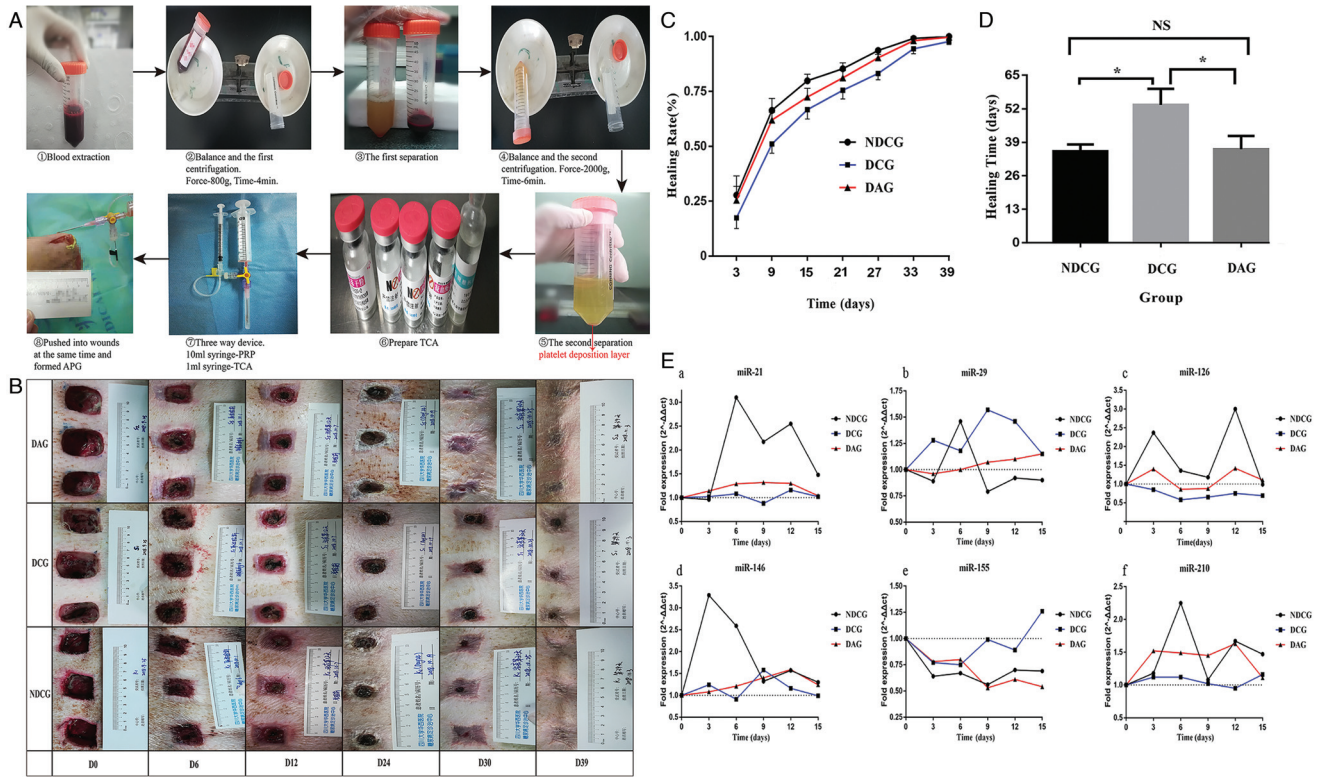

Figure 1: APG in treating diabetic cutaneous wounds in minipigs. (A) Manufacturing process of APG. (B) General view of wounds in different groups at different time points. (C) Holistic comparisons of healing rates in each group. (D) Comparisons of healing time in each group. (E) Dynamic changes of each miRNA in wound tissues in the three groups before and after treatment. * $P < 0.001$. APG: Autologous platelet-rich gel; D0: On the day of treatment; D3/6/9/12/15/21/24/27/30/33/39: 3/6/9/12/15/21/24/27/30/33/39 days after treatment; DAG: Diabetic autologous platelet-rich gel group; DCG: Diabetic control group; NDCG: Non-diabetic control group; NS: No statistics; PRP: Platelet-rich plasma.

wounds in the non-diabetic control group and the diabetic APG group were drier, and the films and scabs easily loosened and fell off. In contrast, scabs in the diabetic control group were closely connected to the wounds. Likewise, the non-diabetic control group and the diabetic APG group grew new epithelium faster than the diabetic control group, so the wounds completely healed earlier in the first two groups [Figure 1B]. Eight different time points of the wound area before and after treatment were used to calculate the healing rates of the three groups. The healing rate of the diabetic APG group was statistically significantly higher than that of the diabetic control group ($P < 0.001$), but was still lower than that of the non-diabetic control group ($P < 0.001$) [Figure 1C]. The healing time of the diabetic APG group was statistically significantly shorter than that of the diabetic control group ($P < 0.001$), and was close to that of the non-diabetic control group ($P = 0.89$) [Figure 1D].

Analysis of miRNA relative expression showed that miR-21, miR-146a, and miR-210 ($P = 0.046$; $P = 0.006$; $P = 0.032$) were increased, and miR-29a and miR-155 ($P = 0.013$; $P = 0.001$) were decreased during the non-diabetic wound healing process, whereas miR-126 ($P = 0.137$) showed no statistically significant differences in time factors. miR-29 and miR-146a ($P < 0.001$; $P = 0.013$) were increased, and miR-126 and miR-155 ($P < 0.001$; $P = 0.018$) were decreased during the diabetic wound healing process, whereas miR-21 and miR-210 ($P = 0.164$; $P = 0.274$) showed no statistically significant differences in time factors. A comparison of the results of the three groups suggested that miR-21 and miR-146a in the diabetic APG group were not statistically significantly increased compared with the diabetic control group ($P = 0.400$, $P = 0.572$), but were statistically significantly lower in the former two groups than the non-diabetic control group ($P < 0.001$, $P = 0.001$, $P < 0.001$, $P < 0.001$). Compared with the diabetic control group, miR-29a and miR-155 were statistically significantly decreased, and miR-210 was statistically significantly increased in the diabetic APG group ($P < 0.001$, $P < 0.001$, $P = 0.004$), while these changes were not statistically significantly different from those in the non-diabetic control group ($P = 0.483$, $P = 1.000$, $P = 0.711$). There were statistically significant differences in miR-126 expression among the three groups. miR-126 was statistically significantly suppressed in the diabetic control group ($P < 0.001$) compared with the non-diabetic control group, while it was statistically significantly upregulated in the diabetic APG group ($P = 0.001$). Figure 1E shows the dynamic changes in the expression of the six miRNAs in the three groups.

Wr-miRNAs play various roles in different phases of wound healing.[3] miR-21 participates in all stages of wound healing, promoting healing by inhibiting wound inflammation, promoting angiogenesis, increasing collagen deposition, and accelerating re-epithelization. miR-146a and miR-155 are closely associated with the inflammatory response. miR-146a inhibits inflammation by decreasing

the expression of pro-inflammatory factors. miR-155 is a proinflammatory factor that can enhance inflammation by activating macrophages. miR-126 and miR-210 are involved in angiogenesis regulation. miR-126 is essential for maintaining the stability and integrity of vascular endothelial cells. miR-210 is a hypoxia-sensitive miRNA that is upregulated in ischemic and hypoxic environments to promote angiogenesis. miR-29 is mainly involved in wound remodeling by inhibiting collagen synthesis, promoting collagen degradation, and reducing matrix protein deposition. The present study proved that hyperglycemia delayed wound healing by decreasing the healing rate and prolonging healing time. We also revealed that compared with non-diabetic wounds, miR-21, miR-126, miR-146a, and miR-210 were statistically significantly decreased, while miR-29 and miR-155 were statistically significantly increased, indicating they may be partially responsible for delayed diabetic wound healing.

Our study suggests that APG reversed the effect of hyperglycemia on wound healing, and we further confirmed this by performing a meta-analysis of randomized controlled trials.[4] Some studies have focused on the mechanism by which APG promotes wound healing, and the common critical factor is that APG releases various bioactive factors after platelet activation. For example, one study found that PRP-Exos (containing microRNAs and other bioactive substances) were most biologically active after platelets were activated by TCA.[5] In this study, we also found that miR-126 and miR-210 were statistically significantly upregulated and miR-29 and miR-155 were statistically significantly downregulated in diabetic wounds treated with APG. Upregulated miR-126 and miR-210 can promote angiogenesis, downregulated miR-155 can inhibit the inflammatory response, and downregulation of miR-29 is conducive to collagen formation. Therefore, these changes may be related to improved wound healing in diabetic wounds treated with APG.

In brief, we demonstrated that hyperglycemia impaired wound healing in Bama miniature pigs and disturbed wr-miRNAs expression in wound tissues. Furthermore, we also confirmed that APG treatment simultaneously promoted diabetic wound healing and partly retroregulated the expression of some wr-miRNAs. Our findings provide a promising therapeutic strategy for the treatment of diabetic cutaneous wounds. We believe that APG or wr-miRNA products can be used as effective adjuvant therapies for diabetic cutaneous wounds. However, further research is needed to explore the deep-rooted relationship between diabetic wound healing and miRNAs, such as their target genes.

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Conflicts of interest

None.

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