



Research Article

EGCG accelerates wound healing in diabetic mice by Notch pathway to enhance epidermis formation

Die Jiang^{1,2,†}, Ming-Ming Li^{1,2,†}, Jian-Fei Li^{1,2,†}, Ye-Wei Huang¹, Cheng-Ting Zi^{1,2}, Xuan-Jun Wang^{1,3}, Jun Sheng^{1,3} (✉), Huan-Huan Xu^{1,4}, Qiang-Qiang Zhu^{1,2} (✉)

¹ Key Laboratory of Pu-er Tea Science, Ministry of Education, Yunnan Agricultural University, Kunming 650201, China

² College of Food Science and Technology, Yunnan Agricultural University, Kunming 650201, China

³ State Key Laboratory for Conservation and Utilization of Bio-Resources in Yunnan, Kunming 650201, China

⁴ College of Science, Yunnan Agricultural University, Kunming 650201, China

[†] Die Jiang, Ming-Ming Li, and Jian-Fei Li contributed equally to this work.

Received: 1 August 2024 / Revised: 19 August 2024 / Accepted: 19 August 2024

Abstract: Keratinocyte proliferation delay was a prominent clinical manifestation of diabetes, significantly impeding the wound healing process in diabetic mice. The potential therapeutic role of (–)-epigallocatechin gallate (EGCG) has been recognized due to its ability to enhance wound healing under diabetic conditions, but the underlying mechanism remained unclear. This study elucidates that EGCG expedited wound healing in diabetic mice by accelerating re-epithelialization and collagen deposition. Remarkably, we observed that EGCG promotes epidermal cell proliferation and enhances wound healing process in diabetic mice. We also discovered an overexpression of the Notch pathway in the epidermal cells of diabetic mice. Interestingly, EGCG effectively suppresses this overexpressed Notch pathway, suggesting a targeted mechanism for its therapeutic effects. Furthermore, the experiments with human immortalized keratinocytes (HaCaT) confirmed that high glucose levels activated the Notch signaling pathway, which was subsequently inhibited by EGCG treatment. In conclusion, our study reveals that EGCG improves wound healing in streptozotocin (STZ)-induced diabetic mice by targeting the Notch pathway in epidermal cells. These findings offered novel insights into therapeutic strategies for diabetic wounds and highlight EGCG as a promising candidate for treating chronic wounds.

Keywords: (–)-epigallocatechin gallate; epidermal; Notch signaling pathway; chronic wounds

Citation: Jiang D., Li M. M., Li J. F., et al. EGCG accelerates wound healing in diabetic mice by Notch pathway to enhance epidermis formation. *Food & Medicine Homology*, 2027, 4: 9420144. <https://doi.org/10.26599/FMH.2027.9420144>

1 Introduction

Diabetes is a rapidly escalating global health issue, affecting approximately 9.5% of the world's population^[1,2]. According to IDF Diabetes Atlas 10th edition, diabetes can potentially lead to complications in various bodily systems and may elevate the risk of premature mortality^[3]. Diabetic foot ulcer (DFU) is one of the complications of diabetes and around one in four people with diabetes will develop into the disease in their lifetime^[3,4]. In addition, diabetic foot issues often result in significant morbidity and mortality, characterized primarily by delayed wound healing^[5,6]. The impact on diabetic wound healing is multifaceted, encompassing impaired growth factor production, collagen accumulation, and keratinocyte migration and proliferation^[5,7]. Therefore, addressing altered wound healing mechanisms could be pivotal in improving DFU outcomes.

Notch signaling, a ubiquitous and evolutionarily conserved intracellular pathway, regulates various cellular functions^[8]. It plays a pivotal role in proliferation, apoptosis/survival, and angiogenesis^[9]. Many studies have demonstrated the relationship between Notch and skin homeostasis. The Notch pathway

balances growth arrest and progressive differentiation processes of keratinocytes^[9,10]. Moreover, it accelerates keratinocyte cell proliferation^[11]. In normal wound healing, Notch signaling positively regulates cell migration and inflammation^[8,12]. However, Notch signaling is overactivated which impairs wound healing in diabetes. Hyperglycemia-induced activation of Notch1 signaling delays keratinocyte cell migration and specifically impairs wound healing in diabetes^[13,14]. These findings suggest that targeting Notch1 signaling could be a potential therapeutic strategy for diabetic wounds.

(–)-Epigallocatechin gallate (EGCG) is regarded as the most abundant compound and an important bioactive component in tea leave^[15]. It exhibits remarkable bioactivities such as antioxidant, free radical scavenging, anti-inflammatory, anti-tumor, anti-obesity, and antimicrobial properties^[15,16] which is under extensive investigation and application in functional foods^[17]. For example, EGCG could applications in oral materials, food packaging, improve the taste of food by related to protein, and prevent food corruption caused by oxidation^[18,19]. EGCG has also been investigated for its application in skin wound treatment, with positive results observed in vivo studies^[13,20]. Notably, EGCG can

exert beneficial effects at various stages of skin wound healing, including hemostasis, inflammation, proliferation, and tissue remodeling^[21]. Our previous study demonstrated that EGCG could enhance wound healing in diabetes^[13]. However, the complex process of skin wound healing and the mechanism of EGCG's role in improving wound healing, especially in diabetes, require further research. Prior studies have identified Notch as a novel receptor for EGCG^[13,22]. Therefore, we hypothesize that EGCG may target Notch to regulate keratinocytes and improve wound healing in diabetes.

In this comprehensive study, we present evidence that elevated glucose levels effectively activate the Notch pathway both *in vitro* and *in vivo*. Moreover, we identify EGCG as a potent agent that significantly enhances wound healing in diabetes by specifically targeting and accelerating keratinocyte proliferation through modulation of the Notch pathway. These crucial findings highlight the therapeutic potential of targeting the Notch pathway combined with EGCG treatment to accelerate epidermis formation and thereby improve wound healing outcomes in diabetes.

2 Materials and methods

2.1 Cell culture and treatment

Human immortalized keratinocytes (HaCaT) were obtained from KCB (Kunming, China). These cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Hyclone, USA) supplemented with high glucose (4,500 mg/L), 10% (V/V) fetal bovine serum (FBS, Biological Industries, USA), and 1% (V/V) penicillin/streptomycin (Biological Industries, USA). They were maintained in an incubator with a room air: CO₂ ratio of 95:5 (V/V) at 37 °C. To assess whether high glucose levels could activate the Notch1 pathway *in vitro* and examine the impact of EGCG on keratinocytes, HaCaT cells were cultured without FBS overnight. Subsequently, these cells were exposed to either 5.5 or 30 mmol/L glucose and treated with PBS (control), *N*-[*N*-(3,5-difluorophenacetyl)-*L*-alanyl]-sphenylglycine *t*-butyl ester (DAPT, 10 μmol/L) was used as a positive group, or EGCG (20 μg/mL) for 24 h. Following this treatment, the cells were collected for Western blotting.

2.2 Animals

Inbred virgin ICR mice (7–8 weeks old, weighing 31.2 ± 2.8 g, male, clean animal) were purchased from CAVENS LAB ANIMAL (SCXK(Su)2021-0013, Changzhou, China). Following a 2-week acclimatization period, the mice were housed in a controlled environment (12 h light/12 h dark cycle; humidity 50%–60%; ambient temperature 24 ± 1 °C) and were administered with standard laboratory food (ShooBree R Rat Food, Nanjing, China) and water *ad libitum*. The animal experiments exclusively utilized male mice. All procedures were conducted in compliance with institutional guidelines at the Yunnan Agricultural University's animal facility and received approval from the Institutional Animal Care and Use Committee (YNAU2023LLWYH008-01, 16, March, 2023). No adverse events were reported.

2.3 Diabetes induction and monitoring

To induce diabetes in mice, we administered a single intraperitoneal injection (i.p.) of 100 mg/kg body weight streptozotocin (STZ) (Sigma, Shanghai, China). The STZ was freshly dissolved in cold citrate buffer (0.05 mol/L; pH 4.5). Prior to STZ injection, all mice were fasted overnight and weighed

(41.1 ± 2.5 g). After overnight fasting, the blood glucose levels (BGLs) of each mouse were measured using tail-vein blood samples with a glucose meter (Sannuo Go with matched strips, Changsha, China). Mice with fasting BGLs > 17 mmol/L (about 306 mg/dL) were confirmed to be diabetic and continued to monitor BGLs^[13,23,24].

2.4 Excisional wounding and treatment

After confirming the diabetes mellitus (DM) mice model for three days, the mice were divided into three treatment groups. Six naïve mice were used as the normal group, and 12 DM mice were randomly assigned to the diabetes group and the EGCG group. One mouse was housed in each cage during the experiment. Mice were administered anesthesia using pentobarbital (30 mg/kg, i.p.), and their dorsal fur was shaved and skin was cleaned with an alcohol swab. Two circular 8-mm full-thickness wounds were created on the back of each mouse with a dermal biopsy punch and the wounds were left uncovered. Group 1: Naïve mice received 1% carboxymethylcellulose (CMC) vehicle during the experiment. Group 2: DM mice were applied with 1% CMC vehicle during the experiment. Group 3: DM mice were treated with 10 μg/mL EGCG^[13] (≥98%, Chengdu Biopurify Phytochemicals Ltd., Chengdu, China) during the experiment. Each group was treated for 14 days, and the wound healing process was evaluated. Treatment was administered at 10 a.m., once daily.

2.5 Assessment of wound healing

As previously described, three blinded reviewers independently verified all wound healing assessments. Mice were anesthetized, and the wound surface area was recorded daily using a digital camera (5D Marker III, Canon, Japan). The straight edges in the photographs served as scales, and the wound area was calculated using NIH ImageJ Software (National Institute of Health, Bethesda, MD, USA). The initial wound size was measured immediately after wounding, and wound closure was assessed over time as a percentage of the initial wound area^[13,25].

2.6 Histochemical staining

On the 8th day post-wound, full-thickness wounds were excised from the dorsum of mice utilizing a 10 mm punch biopsy tool. Subsequently, the excised tissues were fixed in 10% formalin for a duration of 24 h before being embedded in paraffin wax. Sections of 3 μm in thickness were obtained from all the tissue samples and subsequently stained with hematoxylin (Sigma, USA) and eosin (Solarbio, China) to assess the process of re-epithelialization. The extent of re-epithelialization was determined by using the following formula:

$$\text{Extent of re-epithelialization} = \frac{\text{Distance traversed by epithelium over the wound from the wound edge}}{\text{Distance between wound edges}} \times 100$$

This calculation was performed for two sections per wound, and an average value was derived from these sections to represent each individual wound, as described previously^[26,27]. To evaluate collagen deposition, Mason's Trichrome stain (Solarbio, China) was employed on the tissue sections. The percentage area that was stained in each image was then quantified by counting the number of pixels that were distinctly stained above the threshold intensity and normalizing this count to the total pixel count^[13]. Images were captured using a Leica microscope in conjunction with the Leica CellSens Dimension software.

2.7 Immunohistochemical staining

Tissue samples were obtained from the dorsal region of the mice utilizing a 10-mm punch biopsy on the day 8 post-injury. These samples were subsequently fixed in a 10% formalin solution for a duration of 24 h prior to being embedded in paraffin wax. All tissue samples were then sectioned into 3- μ m slices for staining, employing both the ABC kit (VECTOR, USA) and DAB kit (TIANGEN, China) as per the manufacturers' guidelines. The primary antibodies Hes-1 (#11988) and Ki-67 (ab16667) were acquired from Cell Signaling Technology (USA) and Abcam (USA), respectively. In all staining procedures, any samples that exhibited inadequate staining due to technical issues were discarded. Images were captured using Leica microscope and Leica CellSens Dimension software.

2.8 Immunoblot analysis

Cells were lysed using 200 μ L of lysis solution (Beyotime, China) which contained 1 mmol/L phenylmethylsulfonyl fluoride. Whole cell extracts from the tissue were prepared by homogenizing in a protein extraction solution (10 μ L of PBS per mg of wound tissue, Beyotime, China) that also contained 1 mmol/L phenylmethylsulfonyl fluoride. Each well was charged with 60 micrograms of protein, separated using 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and then transferred to polyvinylidene difluoride membranes (Millipore, USA). These membranes were blocked with 5% bovine serum albumin (BSA) and incubated overnight at 4 °C with a primary antibody. They were then incubated for 1 h at room temperature with a horseradish peroxidase-conjugated secondary antibody. The anti-Notch1 antibody [SJ205] (ET1606-55) was purchased from HUABio (CN), while Cleaved-Notch1 (Val1744) (D3B8) (#4147) was purchased from Cell Signaling Technology (USA). Hes-1 (sc-166410) was obtained from SANTA (USA). Anti-rabbit IgG (# HAF008), anti-mouse IgG (# HAF007), and anti-Rat IgG (# HAF005) were all purchased from R&D Systems (USA).

2.9 IHC double staining

For Notch1 and Filaggrin immunohistochemical (IHC) double staining, we utilized 3 μ m skin tissue sections. These were deparaffinized, rehydrated, and underwent antigen retrieval combined with signal detection as previously described^[28]. Sections were incubated in a blocking buffer from the Polymer Double Staining Detection Kit (Mo/HRP + Rb/AP, DS-0003, Beijing Zhongshan Jinqiao Biotechnology Co., Ltd., China) for 60 min, followed by overnight incubation at 4 °C with two primary antibodies: mouse monoclonal anti-Filaggrin (1:200, sc-66192, Santa Cruz Biotechnology, Inc., USA) and rabbit monoclonal anti-Notch1 antibody (1:200 dilution, cat no. ET1606-55, HUABio, Inc., China). After three rinses in TBST for 5 min each, sections were incubated for 1 h at room temperature with HRP- and AP-labeled secondary antibodies premixed in a 1:1 ratio. Following another series of three rinses in TBST for 5 min each, signal detection was performed using 3,3'-diaminobenzidine (DAB) and GBI-Long red dye reagent. This was followed by counterstaining with hematoxylin according to the instructions provided in the Polymer Double Staining Detection Kit manual (Mo/HRP + Rb/AP, DS-0003, Beijing Zhongshan Jinqiao Biotechnology Co., Ltd., China)^[28].

2.10 Statistical analysis

Experimental data are presented as mean $\pm$ SEM. The analysis was performed using one-way ANOVAs via SPSS 19.0 (IBM,

USA) and GraphPad Prism 5 (Graph-Pad Software, Inc., La Jolla, CA, USA). Differences between groups were considered significant if $P \leq 0.05$.

3 Results

3.1 EGCG could accelerated cells migrate under the high glucose experiment

In our previous study, we demonstrated that EGCG could accelerate wound healing^[13]. However, it remains unclear whether EGCG can enhance epidermal cell migration. To confirm that EGCG can accelerate epidermal cell migration, a wound healing assay was applied. The results showed that EGCG could improve cell migration during a 48-h period under a high glucose environment (Figs. 1A and B).

3.2 High glucose levels activate the Notch1 signaling pathway and regulated by EGCG in keratinocytes

The Notch signaling pathway has been confirmed to be a receptor for EGCG and plays a role in cell migration^[29]. To investigate the mechanisms underlying the positive effects of EGCG on wound healing, we evaluated the effect of EGCG on the Notch signaling pathway in keratinocytes. High glucose levels can activate the Notch receptor (Fig. 1C), and the Notch1 intracellular domain (NICD) and downstream target protein Hes-1 also showed enhanced expression (Figs. 1D, E). However, EGCG treatment can regulate the signaling pathway, inhibit Notch1 activation, and decrease Hes-1 expression (Figs. 1C, E). These results demonstrate that Notch can be activated by high glucose levels and regulated by EGCG in keratinocytes.

3.3 Wound healing was significantly improved with EGCG treatment in DM mice

In order to evaluate the effect of EGCG on wound healing in diabetes, we applied EGCG to skin wounds in DM mice. The results showed that wound healing was statistically delayed in diabetic mice compared to the normal group (Fig. 2). However, wound healing was significantly improved with EGCG treatment (Fig. 2). These results suggest that EGCG could improve wound healing in DM mice.

3.4 EGCG accelerate epidermis formation by enhancing keratinocyte proliferation

To assess the impact of EGCG on epidermis formation, hematoxylin-eosin staining was conducted to observe the effects of EGCG on wounded skin structure at day 8. Histological assessment revealed reduced re-epithelialization at the wound edge in STZ-induced DM mice compared to the normal group, and EGCG significantly enhanced re-epithelialization (Figs. 3A and C). Since the extracellular matrix (ECM) contributes to wound healing, Mason's trichrome stain was used to evaluate ECM accumulation. The results showed that EGCG increased collagen deposition, a marker of ECM accumulation, in the wounded skin compared to the DM group (Figs. 3B and D). This suggests that EGCG improves epidermis formation in DM mice.

Furthermore, Ki-67 staining, a marker of cell proliferation, showed a decrease in DM mice compared to the normal group. However, EGCG treatment enhanced epidermal cell proliferation in DM mice (Figs. 3E and F). These findings suggest that EGCG accelerates cell proliferation, particularly in epidermal cells, thereby enhancing epidermis formation.

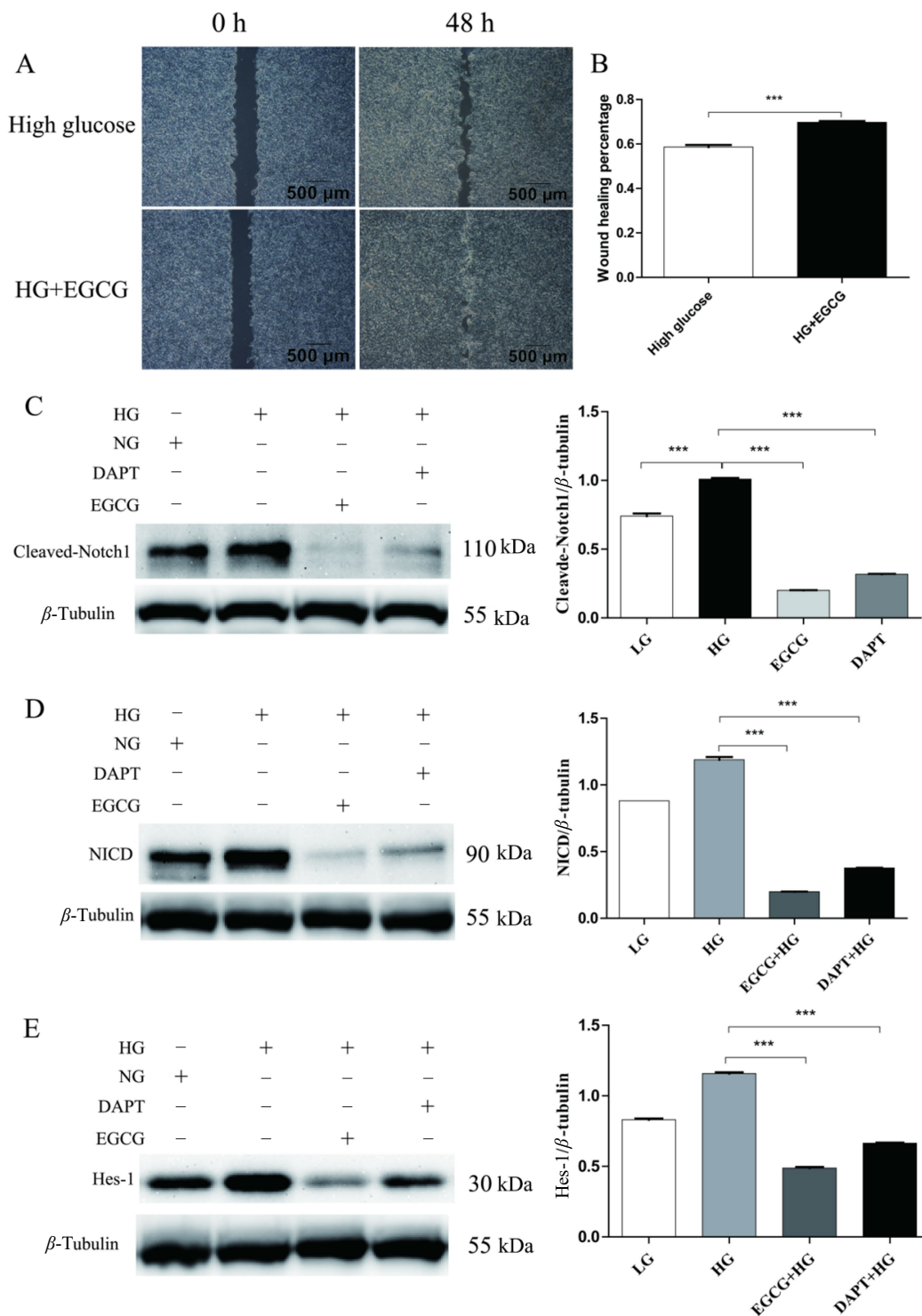


Figure 1 EGCG enhance cell migration in human immortalized keratinocytes by target Notch signaling pathway. To test the effect of EGCG on cell migration, the wound healing assay was apply in the human immortalized keratinocytes (HaCat) using a OLYMPUS inverted microscope (A) and the migration area was measured by NIH ImageJ Software (B). To demonstrate the effect of EGCG on Notch signaling pathway in HACAT, Notch1, NICD, Cleave-Notch1, and Hes-1 were detected by Western blotting (C), and the OD values (D, E, F) were calculated using the ImageJ software (NIH). ***Mean value significantly different from that for high-glucose group, $P < 0.001$. The same below. LG, low-glucose. HG, high-glucose.

3.5 Notch signaling plays a crucial role in epidermis cell proliferation

Notch signaling pathway could regulate cell proliferation, with its function appearing specific to keratinocytes^[14]. Researchers have also identified Notch as a receptor for EGCG^[13]. We next

aimed to assess the impact of EGCG on epidermal cell proliferation through the Notch signaling pathway using immunohistochemistry (IHC) double staining. IHC double staining revealed pronounced Notch1 expression near Filaggrin-positive cells in DM mice, an appearance absent in the normal group and diminished in the EGCG group (Figs. 4A, C). Hes-1, a

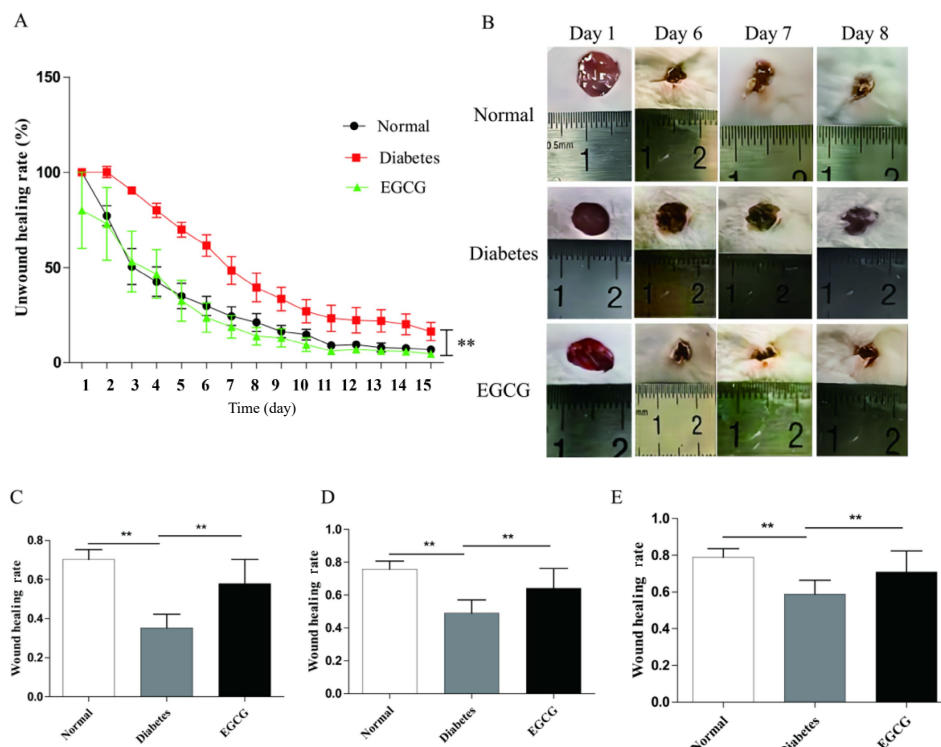


Figure 2 Wound healing in DM mice can be improved by EGCG. Wounds were created using 8-mm punch biopsies on the backs of STZ-induced DM mice and normal mice. Change in wound area was recorded daily using a digital camera and ImageJ (NIH) until complete healing was observed (A). Typical wound healing images were exhibited (B). And showed the changes in wound healing rates at point day 6 (C), day 7 (D), and day 8 (E). Data are pooled from two experiments ($n = 6$ wounds in 6 mice/group, repeated 2 times). Statistical analysis was performed using one-way ANOVA. All data are expressed as mean $\pm$ SEM. **Mean value significantly different from that for diabetes group, $P < 0.01$, ***Mean value significantly different from that for diabetes group, $P < 0.001$. The same below.

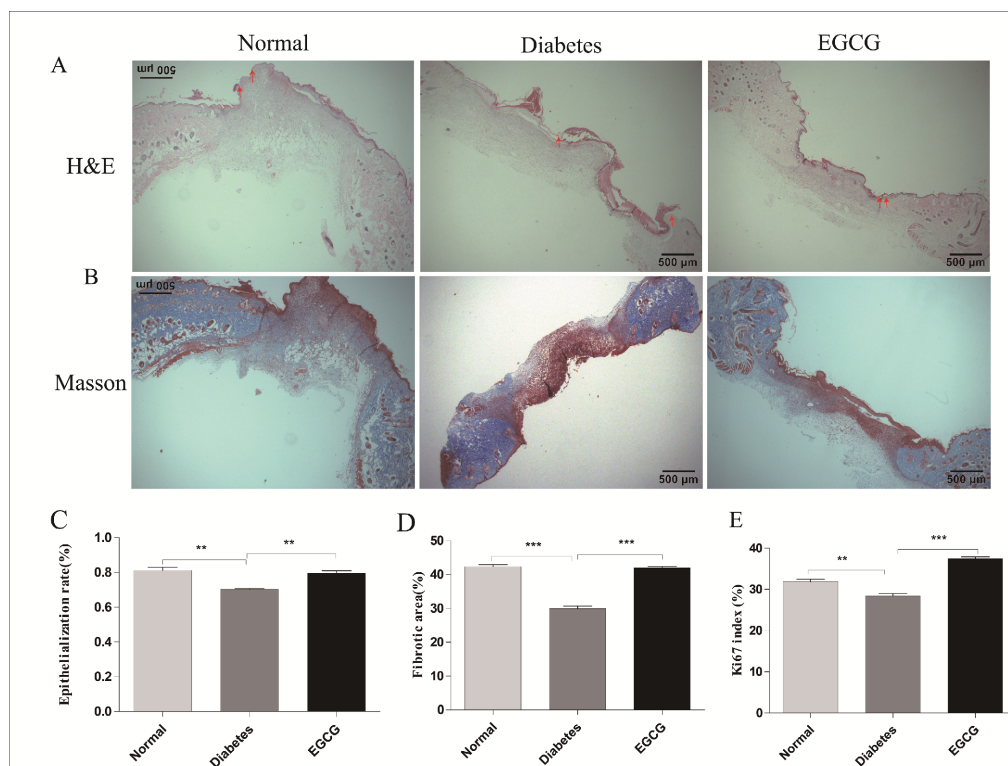


Figure 3 EGCG could accelerate re-epithelialization, collagen deposition, and epidermis cell proliferation. Wounds in DM mice were treated with EGCG or placebo and harvested at day 8 post-injury. Histopathological images showing paraffin sections stained with hematoxylin and eosin (H&E) (A) and collagen deposition (B). The diabetes group shows less re-epithelialization (arrows indicate ends of migrating epithelial) and collagen deposition (blue area) compared with both the normal and EGCG-treated groups. The epithelialization rate (C), fibrotic rate (D), and Ki-67 index (E) was quantification to evaluate the image. IHC was performed on paraffin sections stained for Ki-67. Brown area (red box shown) indicated positive Ki-67 staining. The brown cell as positive area is indicated by arrows, Ki-67 (green arrows) (F). The quantification analysis was calculated using Image pro plus 6.0 software. For all graphs, $n = 5$. Statistical analysis was performed using one-way ANOVA.

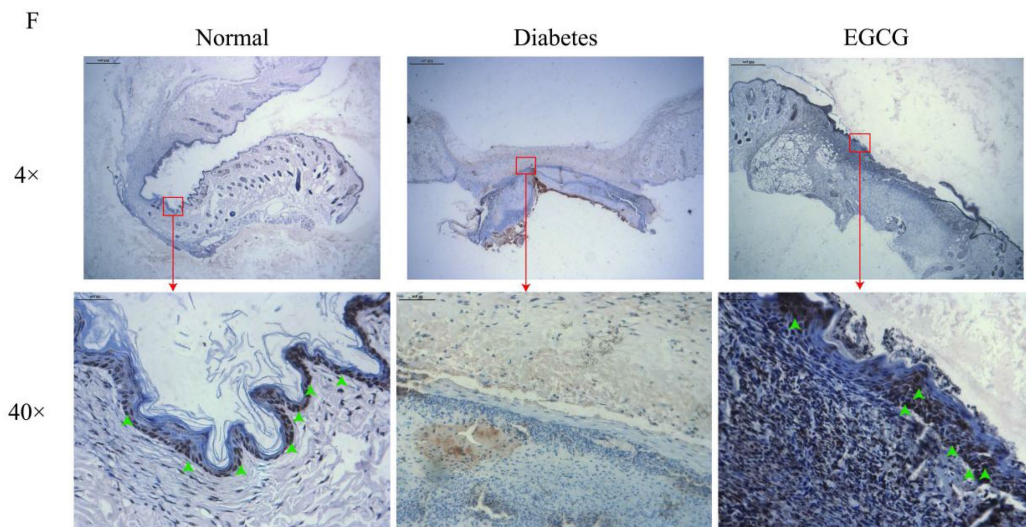


Figure 3 (Continued).

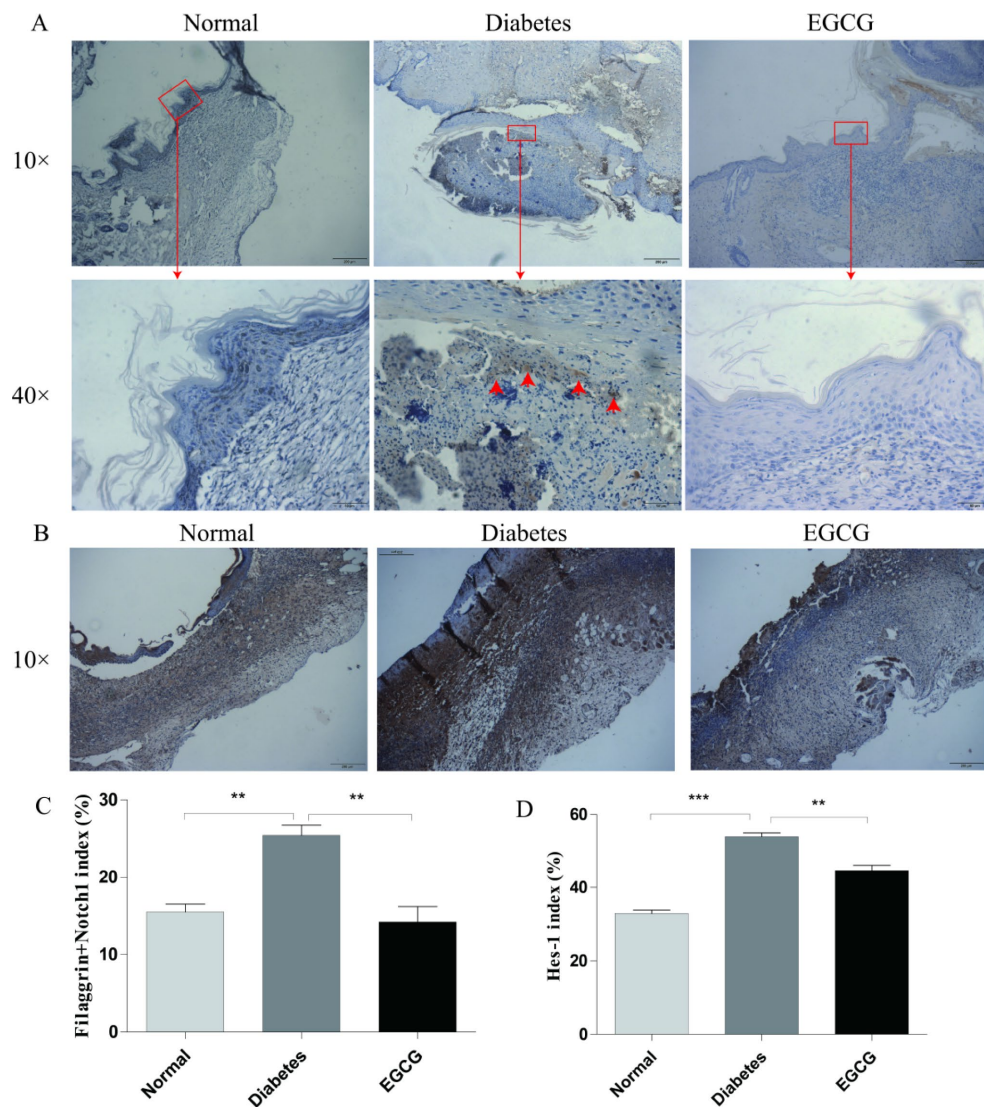


Figure 4 EGCG regulates the Notch signaling pathway in DM epidermis. Wounds in DM mice were treated with EGCG or placebo and harvested at day 8 post-injury. The filaggrin and Notch1 (A) were stained by IHC double staining and the brownish red area (red box shown) indicated filaggrin and Notch1 double-positive. The Hes-1 (B) stained by IHC and brown area (black box shown) indicated positive. The quantification analysis was calculated using Image pro plus 6.0 software. The filaggrin and Notch1 index (C), and Hes-1 index (D) were quantification to evaluate the image. For all graphs, $n = 5$. Statistical analysis was performed using one-way ANOVA.

downstream protein of the Notch signaling pathway, showed that EGCG decreased Hes-1 expression in the epidermis (Figs. 4B, D). This indicates that Notch regulation by EGCG occurs in epidermal cells.

Subsequently, we measured Notch expression levels in the skin to explore the mechanism of EGCG on the Notch signaling pathway. The levels of Notch1 expression were elevated in DM mice skin compared to the normal group, but EGCG arrested the increase in Notch1 expression in DM mice (Fig. 5A). Additionally, the activation of Notch1 (Fig. 5B) and the expression of the downstream protein Hes-1 (Fig. 5C) were reduced by EGCG treatment in DM mice skin. Protein expression levels also demonstrated that the Notch signaling pathway could be modulated by EGCG. These findings underscore the critical role of the Notch signaling pathway in epidermal cell proliferation.

4 Discussion

As one of the most prominent clinical manifestations of diabetes, multiple factors could delay wound healing^[5,30]. Cellular impairment is a major factor that could affect re-epithelialization and dermal remodelling^[31]. Furthermore, keratinocytes at the chronic wound edge show delayed migration *in vitro* and prevent healing in mice^[31]. With the increasing diabetic patient population, the incidence of chronic non-healing wounds is on the rise^[32]. So, novel therapeutics aimed at accelerating keratinocytes migration, enhancing re-epithelialization, and improving wound closure are

urgently needed. In the present study, we observed that EGCG could improve wound healing in diabetic mice through enhances keratinocytes re-epithelialization by targeting Notch. Our results reveal that EGCG can promote epidermis formation in skin wounds via the Notch signaling pathway.

Keratinocytes, the predominant cell type in human skin, are pivotal in the wound healing process^[33]. In diabetic wounds, disruption of various biological mechanisms leads to dysfunction of keratinocytes including impaired migration and proliferation which can lead to poor diabetic wound healing^[33]. Our study revealed that epidermal cell proliferation was inhibited in DM mice, resulting in delayed epithelialization during wound healing (Fig. 3). This finding is consistent with previous research^[14,34]. Several mechanisms have been proposed for the delay in epidermal cell proliferation in DM mice, such as inflammation impairing keratinocyte migration^[35] and high glucose inhibiting the p38 MAPK/Autophagy pathway^[34]. In this study, we identified the Notch pathway as a novel mechanism suppressing epidermal cell proliferation during wound healing in DM mice (Figs. 3, 4), as previously described^[10]. Taken together, keratinocytes play a central role in the epidermis during diabetic wound healing, and their application holds significant potential for treating diabetic wounds. Our study further elucidates this role.

Moreover, the Notch signaling pathway is capable of coordinating the process of epidermal proliferation^[10]. Researchers have reported that the activation of Notch signaling in

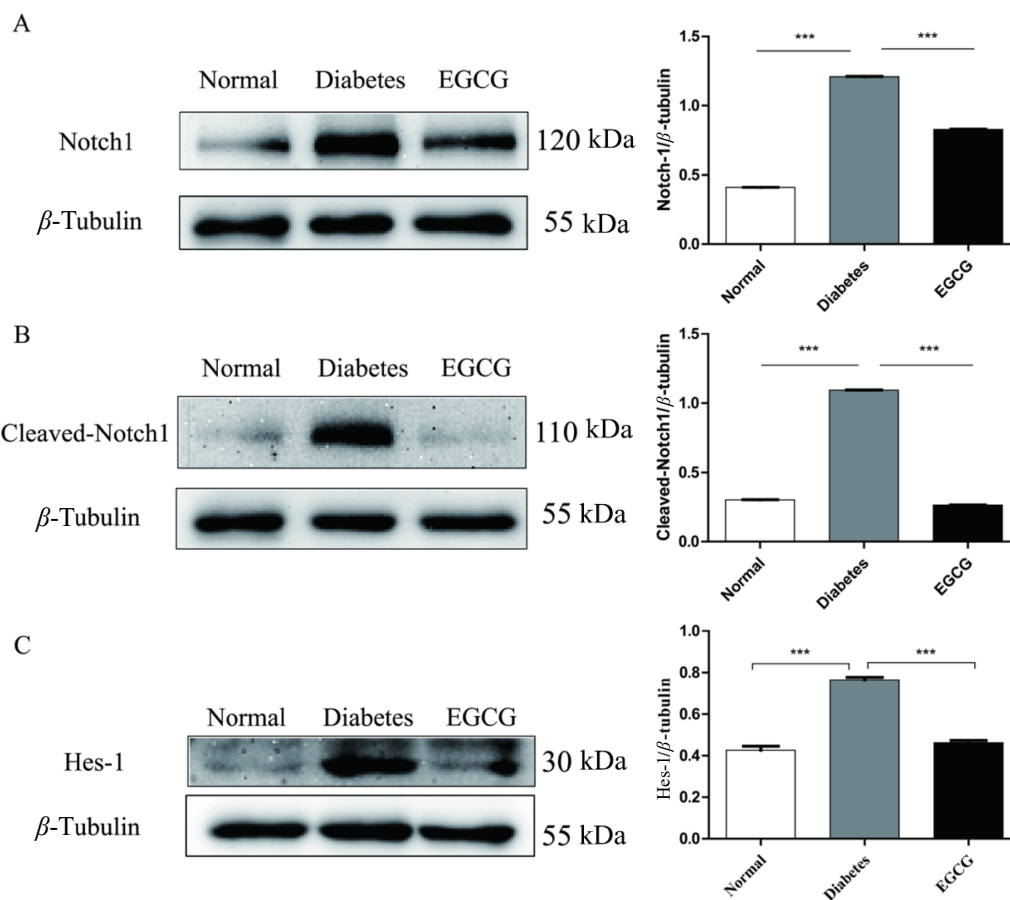


Figure 5 Notch signaling pathway was inhibited by EGCG in DM mice. Tissue was obtained from mice by performing punch biopsies on the backs of animals. And then, the tissue was homogenized in protein extraction solution and 60 µg for each well for detected. The expression of Notch1, Cleaved-Notch1, and the downstream protein Hes-1 (A, B, C) was measured by Western blotting. The OD values (A, B, C) were calculated using ImageJ software (NIH). For all graphs, $n = 5$. Statistical analysis was performed using one-way ANOVA.

hyperglycemia is dependent on the γ -secretase-mediated cleavage of the Notch receptor^[14]. In this study, we demonstrated that Notch can be activated by high glucose levels, and the levels of NICD were increased *in vitro* (Fig. 1). Notch activation was also detected in the skin of DM mice (Fig. 5). The cross-validation of these results with existing literature highlights the significant role of Notch pathways in wound healing in diabetes. Furthermore, this information suggests that Notch could potentially serve as a therapeutic target to enhance epidermal proliferation and epithelization. Previous studies have shown that wound healing under diabetic conditions can be improved by inhibiting Notch signaling^[13]. Similarly, we found that Notch inhibition by EGCG could accelerate epidermis proliferation and improve epithelization (Figs. 2, 3). The wound healing in DM mice can be improved by EGCG through increased epidermis proliferation and inhibition of Notch signaling.

To elucidate the mechanism of EGCG-based enhancement in

wound healing in diabetic mice, we utilized an immortalized human keratinocyte as an experimental model. Consistent with prior research findings^[14], our results indicate that EGCG can suppress high-glucose-induced overactivation of Notch signaling (Fig. 1). Moreover, data from animal experiments reveal that EGCG accelerates epidermal proliferation and inhibits the Notch signaling pathway in the wound skin of diabetic mice (Figs. 4 and 5). However, given that inhibiting inflammation also promotes wound healing through Notch, further investigation is warranted to understand the interplay between epidermis and inflammation *in vivo*.

In conclusion, our study demonstrates that EGCG enhances wound healing by targeting the Notch pathway to promote epithelialization in STZ-induced diabetic mice (Fig. 6). These findings offer a novel perspective on the therapeutic approach for diabetic wounds and suggest EGCG as a novel potential medicine to treat chronic wounds.

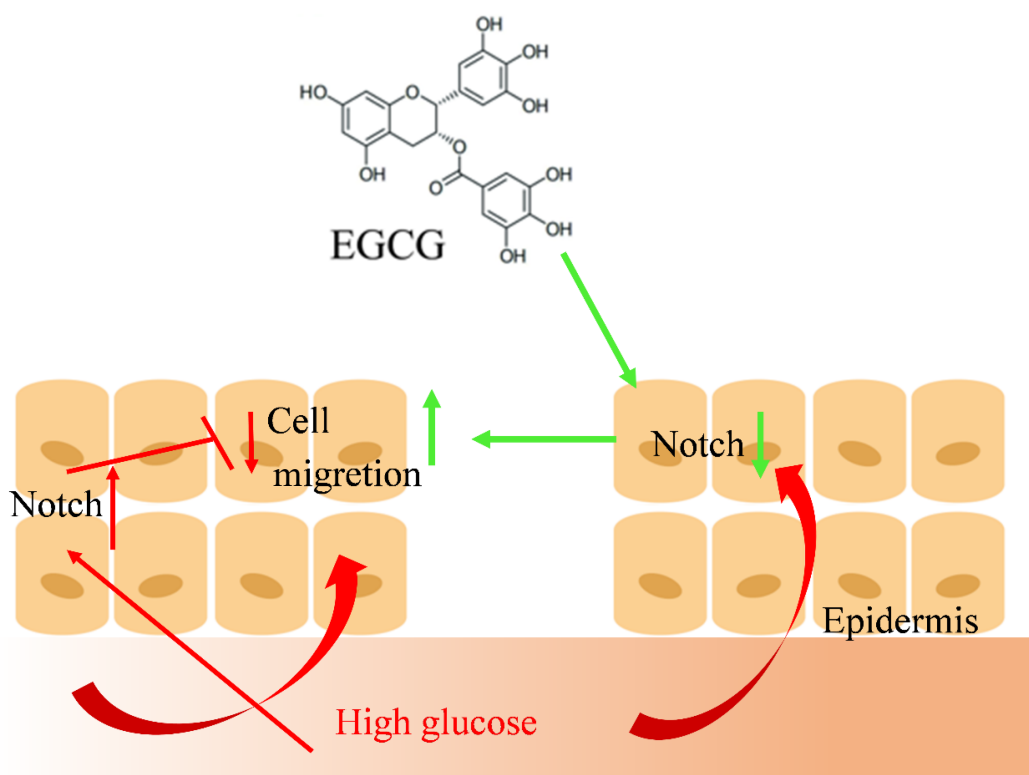


Figure 6 EGCG accelerate epidermis migration by target Notch pathway in DM mice.

Conflicts of interest

The authors declare that there are no conflicts of interest in this work.

Acknowledgments

This study was supported by the Basic Research Project of Yunnan Province (No. 202301AT070505), the Xingdian Talent Plan of Yunnan Province (No. XDYC-QNRC-2023-0427, XDYC-YLXZ-2022-0025). Thanks to Shahai for assisting the English editor.

References

- [1] Williams, R., Karuranga, S., Malanda, B., et al. Global and regional estimates and projections of diabetes-related health expenditure:

results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Research and Clinical Practice*, **2020**, 162: 108072. <https://doi.org/10.1016/j.diabres.2020.108072>

- [2] Hill-Briggs, F., Adler, N. E., Berkowitz, S. A., et al. Social determinants of health and diabetes: a scientific review. *Diabetes Care*, **2021**, 44: 258. <https://doi.org/10.2337/dci20-0053>
- [3] Cole, J. B., Florez, J. C. Genetics of diabetes mellitus and diabetes complications. *Nature Reviews Nephrology*, **2020**, 16: 377–390. <https://doi.org/10.1038/s41581-020-0278-5>
- [4] Monteiro-Soares, M., Russell, D., Boyko, E. J., et al. On behalf of the International Working Group on the Diabetic Foot. Guidelines on the classification of diabetic foot ulcers (IWGDF 2019). *Diabetes/Metabolism Research and Reviews*, **2020**, 36: e3273. <https://doi.org/10.1002/dmrr.3273>
- [5] Davis, F. M., Kimball, A., Boniakowski, A., et al. Dysfunctional wound healing in diabetic foot ulcers: new crossroads. *Current Diabetes Reports*, **2018**, 18: 2. <https://doi.org/10.1007/s11892-018->

0970-z

- [6] Lamont, P., Franklyn, K., Rayman, G., et al. Update on the Diabetic Foot 2012: The 14th Biennial Malvern Diabetic Foot Conference, May 9-11, 2012. *Lower Extremity Wounds*, **2013**, 12: 71–75. <https://doi.org/10.1177/1534734613476519>
- [7] Galkowska, H., Wojewodzka, U., Olszewski, W. L. Chemokines, cytokines, and growth factors in keratinocytes and dermal endothelial cells in the margin of chronic diabetic foot ulcers. *Wound Repair and Regeneration*, **2006**, 14: 558–565. <https://doi.org/10.1111/j.1743-6109.2006.00155.x>
- [8] Chigurupati, S., Arumugam, T. V., Son, T. G., et al. Involvement of Notch signaling in wound healing. *PLoS One*, **2007**, 2: e1167. <https://doi.org/10.1371/journal.pone.0001167>
- [9] Gratton, R., Tricarico, P. M., Moltrasio, C., et al. Pleiotropic role of Notch signaling in human skin diseases. *International Journal of Molecular Sciences*, **2020**, 21: 4214. <https://doi.org/10.3390/ijms21124214>
- [10] Massi, D., Panelos, J. Notch signaling and the developing skin epidermis. *Advances in Experimental Medicine and Biology*, **2012**; pp. 131–141. https://doi.org/10.1007/978-1-4614-0899-4_10
- [11] Zhu, W., Chen, Y., Qu, K., et al. Effects of *Tricholoma matsutake* (Agaricomycetes) extracts on promoting proliferation of HaCaT cells and accelerating mice wound healing. *International Journal of Medicinal Mushrooms*, **2021**, 23: 45–53. <https://doi.org/10.1615/IntJMedMushrooms.2021039854>
- [12] Kimball, A. S., Joshi, A. D., Boniakowski, A. E., et al. Notch regulates macrophage-mediated inflammation in diabetic wound healing. *Frontiers in Immunology*, **2017**, 8: 635. <https://doi.org/10.3389/fimmu.2017.00635>
- [13] Huang, Y. W., Zhu, Q. Q., Yang, X. Y., et al. Wound healing can be improved by (–)-epigallocatechin gallate through targeting Notch in streptozotocin-induced diabetic mice. *The FASEB Journal*, **2018**, 33: 953–964. <https://doi.org/10.1096/fj.201800337R>
- [14] Zheng, X., Narayanan, S., Sunkari, V. G., et al. Triggering of a Dll4–Notch1 loop impairs wound healing in diabetes. *Proceedings of the National Academy of Sciences*, **2019**, 116: 6985. <https://doi.org/10.1073/pnas.1900351116>
- [15] Dai, W., Ruan, C., Zhang, Y., et al. Bioavailability enhancement of EGCG by structural modification and nano-delivery: a review. *Journal of Functional Foods*, **2020**, 65: 103732. <https://doi.org/10.1016/j.jff.2019.103732>
- [16] Hengge, R. Targeting bacterial biofilms by the green tea polyphenol EGCG. *Molecules*, **2019**, 24: 2403. <https://doi.org/10.3390/molecules24132403>
- [17] Mehmood, S., Maqsood, M., Mahtab, N., et al. Epigallocatechin gallate: phytochemistry, bioavailability, utilization challenges, and strategies. *Journal of Food Biochemistry*, **2022**, 46: e14189. <https://doi.org/10.1111/jfbc.14189>
- [18] Chen, Y., Cheng, S., Dai, J., et al. Molecular mechanisms and applications of tea polyphenols: a narrative review. *Food Biochemistry*, **2021**, 45: e13910. <https://doi.org/10.1111/jfbc.13910>
- [19] Liao, S., Tang, Y., Chu, C., et al. Application of green tea extracts epigallocatechin-3-gallate in dental materials: recent progress and perspectives. *Biomedical Materials Research Part A*, **2020**, 108: 2395–2408. <https://doi.org/10.1002/jbm.a.36991>
- [20] Ud-Din, S., Foden, P., Mazhari, M., et al. A double-blind, randomized trial shows the role of zonal priming and direct topical application of epigallocatechin-3-gallate in the modulation of cutaneous scarring in human skin. *Journal of Investigative Dermatology*, **2019**, 139: 1680–1690. <https://doi.org/10.1016/j.jid.2019.01.030>
- [21] Xu, F. W., Lyu, Y. L., Zhong, Y. F., et al. Beneficial effects of green tea EGCG on skin wound healing: a comprehensive review. *Molecules*, **2021**, 26: 6123. <https://doi.org/10.3390/molecules26206123>
- [22] Wang, T., Xiang, Z., Wang, Y., et al. (–)-Epigallocatechin gallate targets notch to attenuate the inflammatory response in the immediate early stage in human macrophages. *Frontiers in Immunology*, **2017**, 8: 433. <https://doi.org/10.3389/fimmu.2017.00433>
- [23] Yoichi Kondo AN, K. H., Mikio I. Low molecular weight chitosan prevents the progression of low doses streptozotocin-induced slowly progressive diabetes mellitus in mice. *Biological and Pharmaceutical Bulletin*, **2000**, 23: 1458–1464. <https://doi.org/10.1248/bpb.23.1458>
- [24] Dekel, Y., Glucksam, Y., Elron-Gross, I., et al. Insights into modeling streptozotocin-induced diabetes in ICR mice. *Lab Anim (NY)*, **2009**, 38: 55–60. <https://doi.org/10.1038/labon0209-55>
- [25] Gallagher, K. A., Joshi, A., Carson, W. F., et al. Epigenetic changes in bone marrow progenitor cells influence the inflammatory phenotype and alter wound healing in type 2 diabetes. *Diabetes*, **2015**, 64: 1420–1430. <https://doi.org/10.2337/db14-0872>
- [26] Qu, H., Miao, T., Wang, Y., et al. Dedicator of cytokinesis 5 regulates keratinocyte function and promotes diabetic wound healing. *Diabetes*, **2021**, 70: 1170–1184. <https://doi.org/10.2337/db20-1008>
- [27] Koike, Y., Yozaki, M., Utani, A., et al. Fibroblast growth factor 2 accelerates the epithelial–mesenchymal transition in keratinocytes during wound healing process. *Scientific Reports*, **2020**, 10: 18545. <https://doi.org/10.1038/s41598-020-75584-7>
- [28] Xu, J., Yu, Y., He, X., et al. Tumor-associated macrophages induce invasion and poor prognosis in human gastric cancer in a cyclooxygenase-2/MMP9-dependent manner. *American Journal of Translational Research*, **2019**, 11: 6040–6054.
- [29] Ding, H., Feng, Z., Hu, K. GRWD1 overexpression promotes the proliferation, migration, and invasion of gastric cancer through the Notch signaling pathway. *Translational Cancer Research*, **2020**, 9: 1–11. <https://doi.org/10.21037/tcr.2019.11.01>
- [30] Guo, S., DiPietro, L. A. Factors Affecting wound healing. *Journal of Dental Research*, **2010**, 89: 219–229. <https://doi.org/10.1177/0022034509359125>
- [31] Wilkinson, H. N., Hardman, M. J. Wound healing: cellular mechanisms and pathological outcomes. *Open Biology*, **2020**, 10: 200223. <https://doi.org/10.1098/rsob.200223>
- [32] Patel, S., Srivastava, S., Singh, M. R., et al. Mechanistic insight into diabetic wounds: pathogenesis, molecular targets and treatment strategies to pace wound healing. *Biomedicine & Pharmacotherapy*, **2019**, 112: 108615. <https://doi.org/10.1016/j.biopha.2019.108615>
- [33] Hosseini Mansoub, N. The role of keratinocyte function on the defected diabetic wound healing. *Clinical Diabetology*, **2022**, 4. <https://doi.org/10.5603/DK.a2022.0004>
- [34] Li, L., Zhang, J., Zhang, Q., et al. High glucose suppresses keratinocyte migration through the inhibition of p38 MAPK/Autophagy pathway. *Frontiers in Physiology*, **2019**, 10: 24. <https://doi.org/10.3389/fphys.2019.00024>
- [35] Huang, S. M., Wu, C. S., Chiu, M. H., et al. High glucose environment induces M1 macrophage polarization that impairs keratinocyte migration via TNF- α : an important mechanism to delay the diabetic wound healing. *Dermatological Science*, **2019**, 96: 159–167. <https://doi.org/10.1016/j.jdermsci.2019.11.004>