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ORIGINAL RESEARCH

METTL3-Mediated m^{6}A Regulation of GDF11 Promotes Socket Healing in Diabetic Rats

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Purpose: Tooth extraction socket healing is impaired in diabetes. We investigated whether the RNA-methyltransferase METTL3 could rescue this defect in an experimental animal study.

Methods: Twelve-week-old male GK and Wistar rats received lentiviral overexpression or knockdown of METTL3 in the extraction socket. Socket healing was evaluated by micro-CT, histology and Quantitative real-time PCR (RT-qPCR). RNA immunoprecipitation (RIP) and dual-luciferase reporter assays were performed to study the interaction between METTL3 and GDF11.

Results: GK rats exhibited significantly lower body weight and METTL3 expression compared to Wistar rats ($p<0.001$). Micro-CT showed a 50% decrease in BV/TV versus diabetic controls ($P < 0.001$), accompanied by lower Tb.N and higher Tb.Sp ($p < 0.001$). Overexpression of METTL3 enhanced tooth extraction socket healing in GK rats, as evidenced by improved bone trabeculae formation and soft tissue healing ($p<0.001$). METTL3 increased GDF11 expression and stability through m^{6}A modification at a specific site ($p<0.001$). Knockdown of GDF11 partially reversed the tooth extraction socket healing effects of METTL3 overexpression ($p<0.01$).

Conclusion: METTL3-mediated m^{6}A methylation of GDF11 enhances socket healing in diabetic rats, identifying METTL3 as a potential therapeutic target for oral wound repair in diabetes.

Keywords: diabetes, tooth extraction socket healing, METTL3, GDF11, m^{6}A modification

Introduction

Socket healing in diabetes is complex and often delayed, leading to prolonged pain, infection, and impaired oral function.[1,2] The International Diabetes Federation reports that 537 million adults lived with diabetes in 2021, and this number is projected to exceed 780 million by 2045.[3] Despite rigorous glycaemic control, diabetic patients still exhibit compromised post-extraction repair, underscoring a critical unmet clinical need.

Recent research has highlighted the role of N^6-methyladenosine (m^{6}A) RNA methylation in various biological processes, including wound healing.[4–6] METTL3, a key m^{6}A methyltransferase, has been implicated in regulating gene expression and cellular functions, making it a potential target for therapeutic interventions in diabetic wound healing.[7,8] However, its contribution to oral wound healing remains largely unexplored.

Intriguingly, growth differentiation factor 11 (GDF11), a member of the TGF-β superfamily, has been shown to play a significant role in tissue regeneration and repair.[9,10] Studies have demonstrated that GDF11 can enhance wound healing by promoting angiogenesis and cell proliferation.[9] Additionally, GDF11 promotes adipogenesis and suppresses osteogenesis by inhibiting m^{6}A modification in an FTO-dependent manner, thereby contributing to the pathogenesis of osteoporosis.[11] This observation raises the possibility that METTL3-mediated m^{6}A modification may directly enhance GDF11 expression to accelerate socket healing in diabetes.

Here, we investigated whether METTL3-dependent epitranscriptomic regulation of GDF11 can rescue impaired socket healing in diabetic rats. Specifically, we aimed to (i) characterise the temporal bone phenotype of diabetic extraction sockets, (ii) elucidate the mechanistic link between METTL3 and GDF11 in this context, and (iii) determine

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whether METTL3 overexpression can functionally restore socket repair in vivo. By elucidating the mechanisms through which METTL3 and GDF11 interact, we hope to identify novel therapeutic targets for improving tooth extraction socket healing in diabetic patients.

Materials and Methods

Experimental Animals

This study was approved by the Experimental Animal Welfare Ethics Committee of Qingdao Harwars (Approval Number: IACUC Issue No:AUP-QY-C-S-2025-003). All animal experiments were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals. Additionally, all methods were carried out in accordance with the ARRIVE guidelines for the reporting of in vivo experiments. Twelve-week-old male Goto-Kakizaki (GK) and Wistar rats were purchased from Changzhou Cavens Experimental Animal Co., Ltd. and Charles River Laboratories, respectively. Before being housed in a specific pathogen-free environment, the rats were allowed a one-week acclimatization period.

Dental Extraction Procedure

Following anesthesia induction, bilateral maxillary first molars were extracted from all rats. Briefly, the oral cavity was gently opened using a sterile retractor, and the gingival tissue surrounding the first molars was carefully dissected to expose the tooth roots. A sterile dental elevator was used to loosen the periodontal ligament, and the teeth were subsequently extracted using forceps. Hemostasis was achieved by applying gentle pressure with sterile gauze. Postoperative care included monitoring for signs of pain or infection, and rats were provided with soft food to facilitate recovery. Pain was monitored daily for 7 d using a 4-point behavioural score; infection was defined by purulent discharge, swelling, or fever >38.5 °C.

Injection of Lentivirus Into Dental Extraction Sites

After dental extraction, GK rats were administered lentiviral particles overexpressing METTL3 (LV-METTL3) or lentiviral particles downregulating GDF11 (LV-shGDF11) at the extraction sites (Vigene Biosciences). Briefly, the rats were anesthetized, and the oral cavity was exposed using a sterile retractor. Lentivirus (4×10^8 TU/mL) was delivered at a final dose of 4×10^6 TU per socket via a 10 μL micro-syringe (Hamilton 1701 RN, 26-gauge needle) in a total volume of 10 μL. Rats were monitored post-injection for any signs of discomfort or adverse reactions. This procedure was performed under aseptic conditions to minimize the risk of infection. Control groups received injections of lentiviral particles carrying a negative control vector (Lv-NC or LV-shNC) under identical conditions. 21 days after tooth extraction and injection, rats in each group were euthanized for cervical dislocation after anesthesia.

Micro-CT Analysis

The bone morphology within the tooth extraction socket at days 21. The harvested specimens were scanned with micro-CT (SCANCO Medical AG) in high resolution using a tube current of 200 μA, voltage of 90 kV, and slice thickness of 20 μm. The maxillary specimens were reconstructed from two- and three-dimensional images with the center of the extraction socket defect as the region of interest. The percentage of bone present in the tooth extraction sockets was calculated using the bone volume fraction (bone volume divided by total tissue volume, BV/TV) and, trabecular number (Tb.N), trabecular thickness (Tb.Th), and trabecular separation (the distance between the two bony trabeculae, Tb.Sp).

Quantitative Real-Time PCR Analysis

Total RNA was extracted from rat serum using the TRIzol reagent (Invitrogen) according to the manufacturer's instructions. RNA concentration and purity were determined. First-strand cDNA was synthesized, and the reaction was performed at 37°C for 15 min, followed by 85°C for 5s to inactivate the reverse transcriptase. Quantitative real-time PCR (RT-qPCR) was performed using the SYBR Green PCR Master Mix (Applied Biosystems) on a QuantStudio 6 Flex Real-Time PCR System (Thermo Fisher Scientific). The thermal cycling conditions were as follows: initial denaturation at 95°C for 10 min, followed

by 40 cycles of 95°C for 15s and 60°C for 1 min. Relative gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method, with GAPDH as the internal reference gene. The primer sequences used for RT-qPCR are listed below: Rat METTL3: Forward 5′-GTTGACCTGGAGATAGAAAGT-3′, Reverse 5′-GATAATCCGTCTGAAGTGC-3′. Human METTL3: Forward 5′-TTGTCTCCAACCTTCCGTAGT-3′, Reverse 5′-CCAGATCAGAGAGGTGGTGTAG-3′. Rat WTAP: Forward 5′-TAAAGCAGCAACAGCAGG-3′, Reverse 5′-GCGTAAACTTCCAGGCAC-3′. Rat FTO: Forward 5′-GACACTTGGCTTCCTTACC-3′, Reverse 5′-CGGCACAGCGTCTTCATT-3′. Rat ALKBH3: Forward 5′-ATCCTCACTGGCTTCCTG-3′, Reverse 5′-CACTGTCCTTCTCGTCCC-3′. Rat ALKBH5: Forward 5′-CTTTAGCGACTCGGCACTT-3′, Reverse 5′-CTCATCAGCAGCATACCCAC-3′. Rat GDF11: Forward 5′-CAAACTGCGGCTCAAGGA-3′, Reverse 5′-GCTGAAGTGGAAATGGCAAC-3′. Human GDF11: Forward 5′-TCTTGCGACTAAAACCCCTAAC-3′, Reverse 5′-GTGAGCGGATACGGATGTGAC-3′. Rat GAPDH (internal control): Forward 5′-GCAAGTTCAACGGCACAG-3′, Reverse 5′-GCCAGTAGACTCCACGACAT-3′. Human GAPDH (internal control): Forward 5′-AATGGACAACTGGTCGTGGAC-3′, Reverse 5′-CCCTCCAGGGGATCTGTTTG-3′.

RNA Stability Detection

To assess the mRNA stability of GDF11, cells were exposed to 10 μg/mL Actinomycin D (Selleck) for durations of 0, 4, 8, and 12 h.[12] Following the respective treatment periods, the mRNA expression levels of GDF11 were quantified using RT-qPCR, as described in the previous section.

HE Staining

Following dental extraction, maxillary bone tissues containing the extraction sockets were harvested from Wistar rats and fixed in 4% paraformaldehyde for 24 h at 4°C. The tissues were decalcified in 10% EDTA solution (pH 7.4) for 4 weeks, dehydrated through a graded ethanol series, cleared in xylene, and embedded in paraffin. For H&E staining, slides were deparaffinized in xylene, rehydrated through graded ethanol, and stained with hematoxylin for 5 min. After rinsing, sections were differentiated in 1% acid alcohol, blued in 1% ammonia water, and counterstained with eosin for 2 min. Slides were then dehydrated, cleared in xylene, and mounted with neutral balsam. Stained sections were examined under a light microscope (Zeiss), and images were captured for analysis.

Cell Culture

HEK293T cells purchased from ATCC (CRL-3216) were cultured in DMEM (Gibco) supplemented with 10% FBS and 1% penicillin-streptomycin at 37°C in a humidified atmosphere with 5% CO_2. Cells were passaged every 2–3 days upon reaching 80–90% confluence by detaching them with 0.25% trypsin-EDTA, followed by resuspension in fresh medium and seeding at a 1:5 split ratio. For lentiviral transfection of HEK293T cells to overexpress METTL3, cells were seeded in a 6-well plate at a density of 2×10^5 cells per well and cultured overnight in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin. The lentiviral particles (4×10^8 TU/mL) carrying the METTL3 overexpression construct were added to the cells at a multiplicity of infection of 10 in the presence of 8 μg/mL polybrene to enhance transduction efficiency. After 24 h, the medium was replaced with fresh complete medium, and cells were further cultured for 72 h.

Evaluation of m^6A Levels

Total RNA was extracted 72 h post-transfection using TRIzol reagent (Vazyme), and mRNA was purified using a Poly(A) mRNA Isolation Kit (Thermo Fisher Scientific). The m^6A levels of GDF11 mRNA were quantified using an m^6A RNA methylation assay kit (EpiQuik™ m^6A RNA Methylation Quantification Kit) according to the manufacturer's instructions. Briefly, 200 ng of purified mRNA was coated onto assay wells, followed by incubation with an anti-m^6A antibody and a detection antibody. The absorbance was measured at 450 nm using a microplate reader (TECAN), and the m^6A levels were normalized to the total RNA input.

RNA Immunoprecipitation

To detect GDF11 levels enriched on METTL3 antibody, an RNA immunoprecipitation assay was performed. HEK293T cells were lysed in RIP buffer, and the lysate was pre-cleared with protein A/G beads. The supernatant was divided into three aliquots:

one for input (10% of total lysate), one for immunoprecipitation with METTL3 antibody, and one for control immunoprecipitation with IgG. The lysates were incubated with METTL3 antibody or IgG overnight at 4°C, followed by the addition of protein A/G beads for 2 h. The beads were washed extensively with RIP buffer, and the bound RNA was extracted using TRIzol reagent. GDF11 mRNA levels in the input, METTL3 antibody, and IgG groups were quantified by RT-qPCR using specific primers. The relative enrichment of GDF11 was calculated by normalizing to the input and comparing the METTL3 group to the IgG control.

Double Luciferase Reporter Assay

The m^6A methylation sites of GDF11 were predicted using the SRAMP database (http://www.cuilab.cn/sramp).[13,14] The wild-type and mutant GDF11 fragments were cloned into the pmirGLO reporter vector using Spe I and Hind III restriction sites, generating GDF11-WT and GDF11-MUT constructs, respectively. These reporter plasmids were co-transfected with oe-nc or oe-FTO into HEK293T cells using Lipofectamine 3000 (Invitrogen). After 48 h, cells were harvested and lysed, and firefly luciferase activity was measured and normalized to renilla luciferase activity. This assay evaluated the regulatory effect of METTL3 on GDF11 expression.

Data Analysis

Statistical analysis was performed using GraphPad Prism 8.0 (GraphPad Software, USA). Each rat contributed one socket; thus, n represents the number of animals, and all statistical tests were performed per animal. Normality was verified with the Shapiro–Wilk test; data that passed the normality test were analyzed by Student's *t*-test (for two groups) or one-way ANOVA followed by Tukey's post-hoc test (for multiple groups), while non-parametric data were analyzed using the Mann–Whitney *U*-test or Kruskal–Wallis test with Dunn's correction. A p-value < 0.05 was considered statistically significant.

Results

Significant Differences in Body Weight and METTL3 Expression Between GK and Wistar Rats Post-Tooth Extraction

The body weight of GK rats, a non-obese model of type II diabetes, was significantly lower compared to Wistar rats at all time points following dental extraction (0, 7, 14, and 21 days) ($p<0.001$, Figure 1A). While both groups exhibited a gradual increase in body weight over time, the GK rats consistently maintained a lower body weight, reflecting the metabolic disturbances associated with type II diabetes. To clarify the time course of glycaemic changes after tooth extraction, we measured fasting blood glucose at 0 (before extraction), 3, 7, 14 and 21 days in both GK and Wistar rats. GK rats remained hyperglycaemic at all time points, with blood glucose levels of 20.2 ± 1.8 mmol/L (0 d), 20.5 ± 2.1 mmol/L (3 d), 19.9 ± 1.9 mmol/L (7 d), 20.3 ± 2.0 mmol/L (14 d) and 20.4 ± 1.7 mmol/L (21 d). Wistar controls maintained normoglycaemia: 5.4 ± 0.5, 5.5 ± 0.6, 5.3 ± 0.4, 5.6 ± 0.5 and 5.4 ± 0.5 mmol/L, respectively ($p<0.001$, Figure 1B). The results indicated no significant difference in blood glucose levels before and after tooth extraction within each group. However, there was a statistically significant difference in blood glucose levels between the two groups both before and after the procedure. The dental extraction procedure did not significantly alter blood glucose levels in either group. Then we detected the expression levels of METTL3, WTAP, FTO, ALKBH3 and ALKBH5 in the serum of the two groups of rats, in order to reveal the dynamic changes of m^6A modification in diabetes. The results suggested that METTL3 levels were significantly lower in the GK group compared with Wistar control group ($p < 0.001$, Figure 1C).

Overexpression of METTL3 Enhances Tooth Extraction Socket Healing in Diabetic GK Rats

To further investigate the role of METTL3 in tooth extraction socket healing, we constructed LV-METTL3 and control plasmids LV-NC. The qPCR analysis results suggested that METTL3 levels were significantly higher in LV-METTL3 group compared with the LV-NC group ($p < 0.001$, Figure 2A). These plasmids were injected into the tooth extraction sites of GK rats, which were then divided into four groups: Wistar control, GK, GK injected with LV-NC, and GK injected with LV-METTL3. After 21 days of tooth extraction in Wistar rats, HE staining showed significant formation of

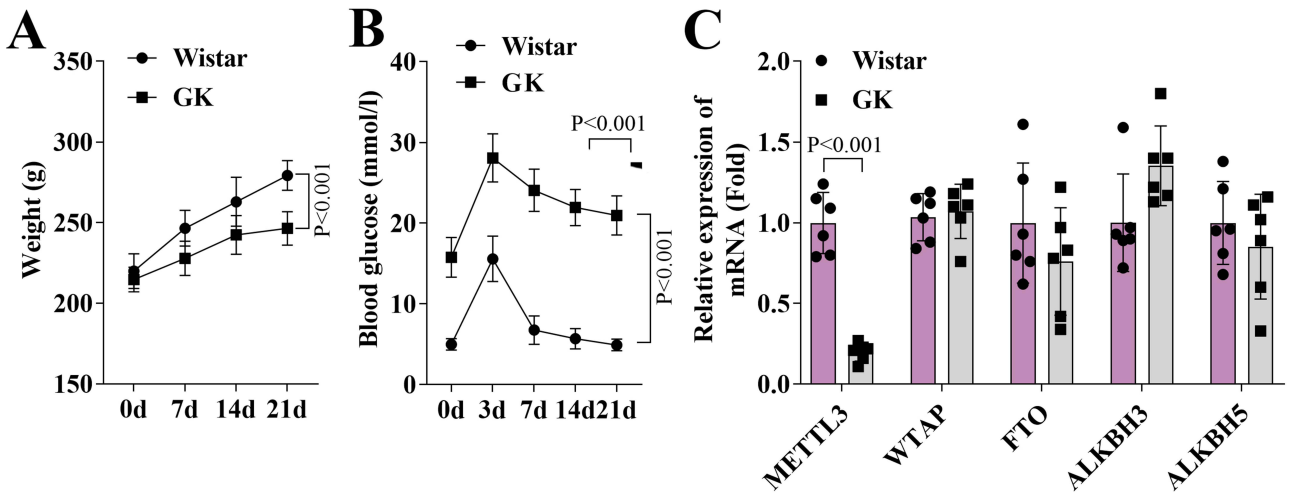


Figure 1 Body weight and m^{6}A-related gene expression in GK and Wistar rats after tooth extraction. (**A**) Body weight changes in GK and Wistar rats measured every 7 days post-tooth extraction, n = 6 rats per group. Data are presented as mean ± SD. p<0.001 indicates a statistically significant difference between the two groups. (**B**) Blood glucose changes in GK and Wistar rats measured at 0, 3, 7, 14, and 21 days post-tooth extraction, n = 6 rats per group. Data are presented as mean ± SD. p<0.01 indicates a statistically significant difference between the two groups. (**C**) Relative expression levels of m^{6}A-related genes (METTL3, WTAP, FTO, ALKBH3, and ALKBH5) in serum samples from GK and Wistar rats 7 days post-extraction. METTL3 expression was significantly lower in GK rats compared to Wistar controls, n = 6 rats per group. Data are presented as mean ± SD. p<0.001 indicates a statistically significant difference between the two groups.

new bone trabeculae in the extraction socket, good soft tissue healing, and absorption of blood clots. However, socket healing in GK rats was poor, whereas LV-METTL3 markedly improved bone fill and epithelial closure (Figure 2B). The BV/TV and Tb.N of the tooth extraction socket were significantly 50% lower in the GK group than in the Wistar group, and Tb.Sp was prominently higher in the GK group, but Tb.Th did not differ significantly between the two groups (p < 0.001, Figure 2C–F).

METTL3 Enhances GDF11 Expression and Stability Through m^{6}A Modification

GDF11 plays an important role in tooth extraction socket healing of diabetes.[9] To elucidate the mechanism by which METTL3 regulates GDF11, we overexpressed METTL3 (p < 0.001, Figure 3A) in HEK293T cells and observed a significant increase in the m^{6}A modification level of GDF11 (p < 0.001, Figure 3B). Concurrently, the mRNA level of GDF11 was also elevated upon METTL3 overexpression (p < 0.001, Figure 3C). RNA immunoprecipitation experiments confirmed that GDF11 was significantly enriched in METTL3 antibody pulldowns, indicating a direct interaction between METTL3 and GDF11 (p < 0.001, Figure 3D). Using the SRAMP online database, we predicted four potential m^{6}A modification sites on GDF11 with high confidence scores (Figure 3E and F). We then performed site-directed mutagenesis on these sites and used a dual-luciferase reporter assay to measure changes in luciferase activity before and after METTL3 overexpression. The results revealed that METTL3 overexpression significantly increased the luciferase activity at site 2, while mutations at this site abolished this effect. In contrast, METTL3 overexpression did not affect the luciferase activity at the other sites, suggesting that METTL3 specifically regulates GDF11 through site 2 (p < 0.001, Figure 3G–J). Additionally, METTL3 overexpression significantly reduced the degradation of GDF11 mRNA, further supporting its role in stabilizing GDF11 transcripts (p < 0.01, Figure 3K).

GDF11 Knockdown Partially Reverses METTL3-Mediated Tooth Extraction Socket Healing in Diabetic GK Rats

To further investigate the role of GDF11 in the tooth extraction socket healing process mediated by METTL3, we performed in vivo knockdown of GDF11 in GK rats. The rats were divided into six groups: Wistar control, GK, GK injected with LV-NC, GK injected with LV-METTL3, GK injected with LV-METTL3 and LV-shNC, and GK injected with LV-METTL3 and LV-shGDF11. The relative expression of GDF11 was measured to confirm the knockdown efficiency (p < 0.001, Figure 4A). The HE staining results suggested that inhibition of GDF11 significantly reversed

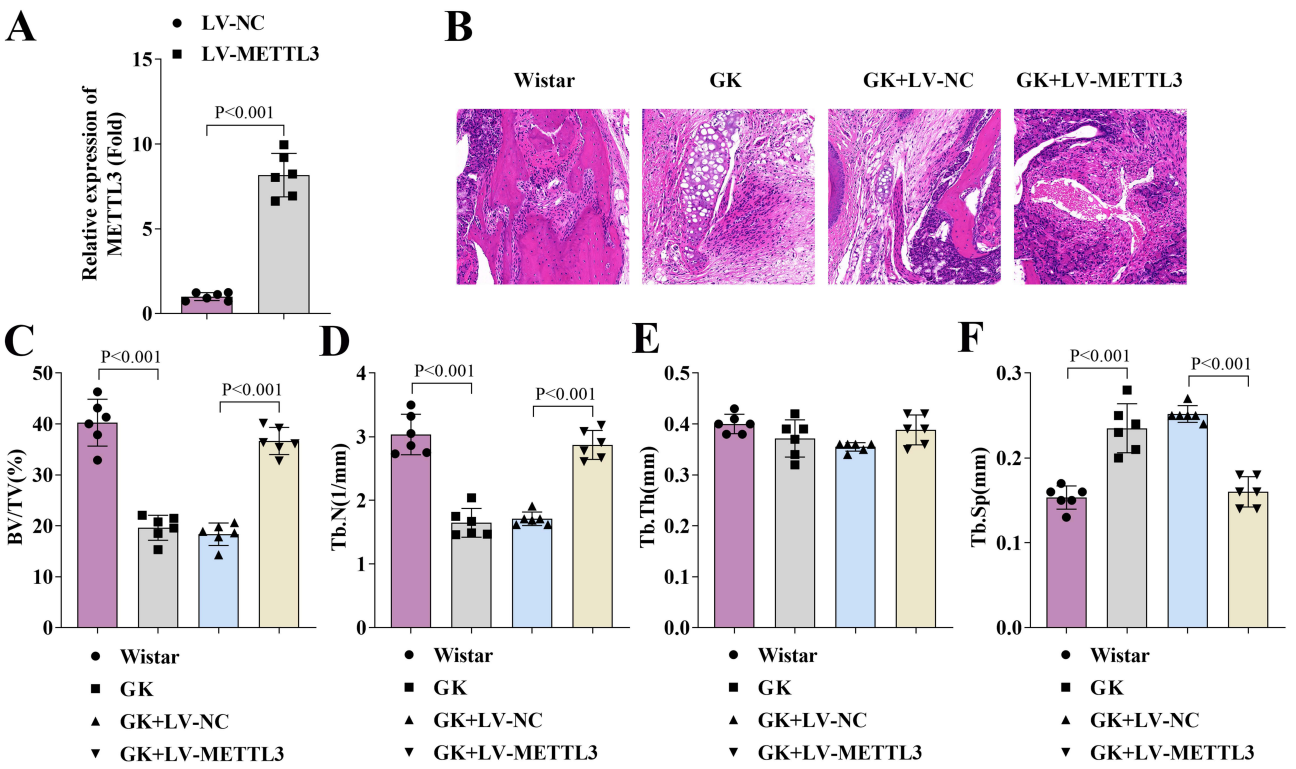


Figure 2 Overexpression of METTL3 enhances tooth extraction socket healing in GK rats. (**A**) Relative expression of METTL3 in tooth extraction socket of GK+LV-NC and GK+LV-METTL3 rats, n = 6 sockets per group (one socket per rat). Data are presented as mean ± SD. $p<0.001$ indicates a statistically significant difference between the GK+LV-METTL3 group and the GK+LV-NC group. (**B**) Representative images of HE staining showing tooth extraction socket healing at the tooth extraction sites in Wistar control, GK, GK+LV-NC, and GK+LV-METTL3 groups, n = 6 sockets per group (one socket per rat). (**C–F**) BV/TV, Tb.N, Tb.Th, and Tb.Sp in the extraction socket 21 days after tooth extraction, n = 6 sockets per group (one socket per rat). Data are presented as mean ± SD. $p<0.001$ indicates a statistically significant difference between groups.

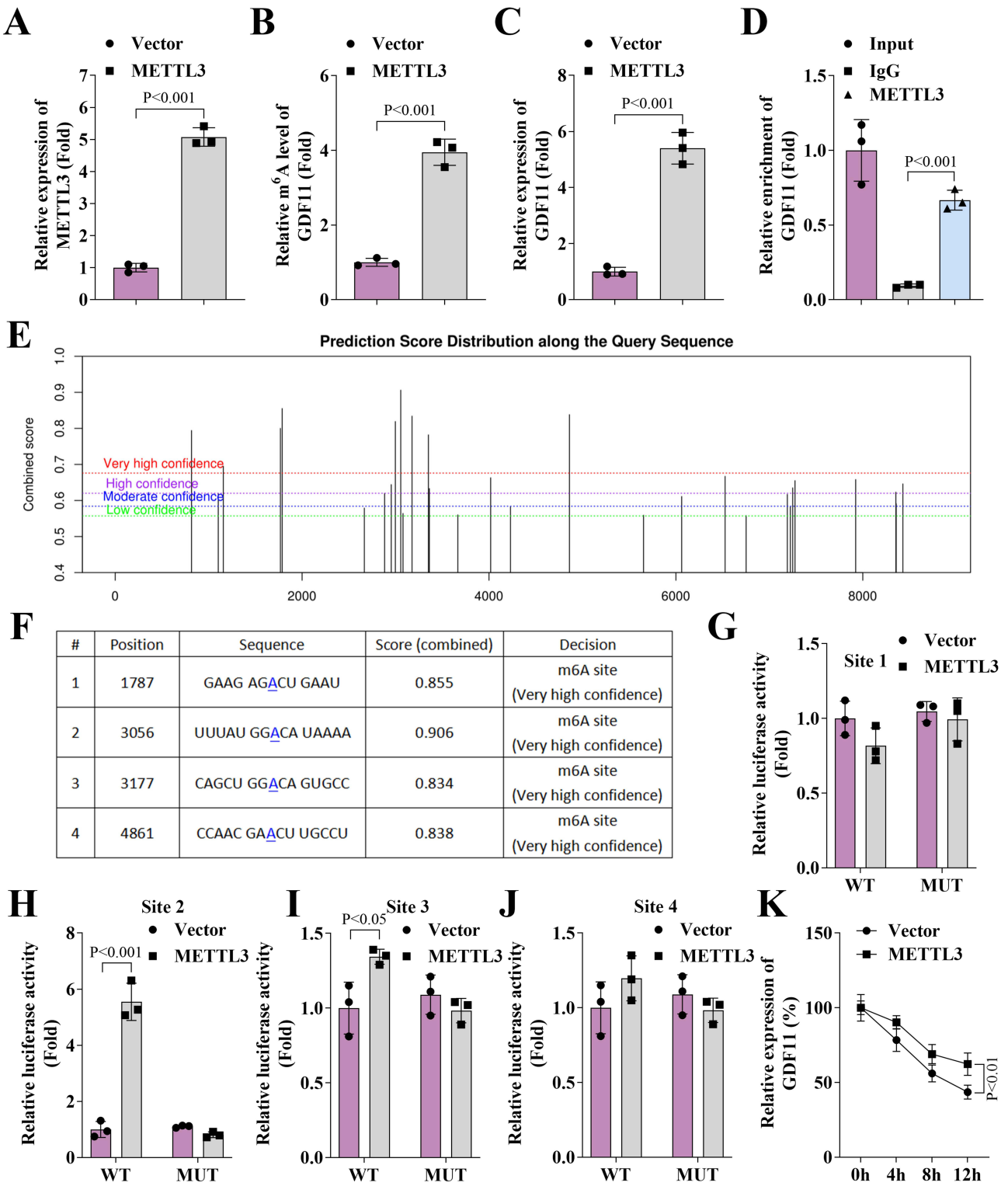
the effects of overexpression of METTL3 on tooth extraction socket healing of extraction socket by inhibiting tooth extraction socket healing (Figure 4B). Moreover, the BV/TV and Tb.N decreased in the GK group were upregulated by overexpression of METTL3, and Tb.Sp significantly upregulated in the GK group was downregulated by overexpression of METTL3. Inhibition of GDF11 significantly reversed the effects of METTL3 overexpression on regulating BV/TV, Tb.N, and Tb.Sp levels. Tb.Th did not differ significantly between the six groups ($p < 0.001$, Figure 4C–F).

Discussion

The findings of this study provide significant insights into the role of METTL3-mediated m^6A modification in diabetic tooth extraction socket healing. Our results demonstrate that METTL3 overexpression enhances tooth extraction socket healing in diabetic GK rats by increasing the expression and stability of GDF11 through specific m^6A modifications. This is consistent with previous studies that have highlighted the importance of m^6A modifications in regulating gene expression and cellular functions.[15,16]

Chronic wounds in diabetes remain clinically vexing. Recent studies have found that local application of GDF11 can promote the healing of skin wounds in type 1 diabetes and type 2 diabetes mice.[9] At the molecular level, GDF11 can significantly upregulate the expression of hypoxia inducible factor-1 α, thereby enhancing the activity of vascular endothelial growth factor and vascular endothelial cell growth factor-1 α, and promoting angiogenesis. This finding supports the potential of GDF11 as a non healing therapeutic agent for diabetes. In addition, a study have shown that there are differences in m^6A modification between diabetes BMSCs and normal BMSCs, and GDF11 candidate genes have been identified.[17] Therefore, we speculated that GDF11 can be m^6A modification to participate in the progress of diabetic tooth extraction socket healing.

In this study, we found that METTL3 expression was aberrant downregulated in GK rats after tooth extraction, and this is in line with previous studies.[7,18] The observed improvement in tooth extraction socket healing following METTL3



#	Position	Sequence	Score (combined)	Decision
1	1787	GAAG AGACU GAAU	0.855	m6A site (Very high confidence)
2	3056	UUUAU GGACA UAAAA	0.906	m6A site (Very high confidence)
3	3177	CAGCU GGACA GUGCC	0.834	m6A site (Very high confidence)
4	4861	CCAAC GAACU UGCCU	0.838	m6A site (Very high confidence)

Figure 3 METTL3 regulates GDF11 through specific m^{6}A modification sites. (**A**–**C**) HEK293T cells transduced with METTL3-overexpressing or control lentivirus, n = 3 independent experiments. (**A**) Relative expression of METTL3 mRNA, (**B**) GDF11 m^{6}A modification levels and (**C**) GDF11 mRNA in HEK293T cells overexpressing METTL3. Data are presented as mean ± SD. $p<0.001$ indicates a statistically significant difference between two groups. (**D**) RIP assay showing the enrichment of GDF11 in METTL3 antibody pulldowns, n = 3 independent experiments. Data are presented as mean ± SD. $p<0.001$ indicates a statistically significant difference compared to the IgG control. (**E**) Prediction of m^{6}A modification sites on GDF11 using the SRAMP database. (**F**) Four high-confidence sites were identified. (**G**–**J**) Dual-luciferase reporter assay showing the effect of METTL3 overexpression on luciferase activity at predicted m^{6}A sites, n = 3 independent experiments. Data are presented as mean ± SD. $p<0.001$ indicates a statistically significant difference compared to the control. (**K**) GDF11 mRNA in HEK293T cells overexpressing METTL3 treated with Actinomycin D at 0, 4, 8, and 12 h, n = 3 independent experiments. Data are presented as mean ± SD. $p<0.01$ indicates a statistically significant difference compared to the control.

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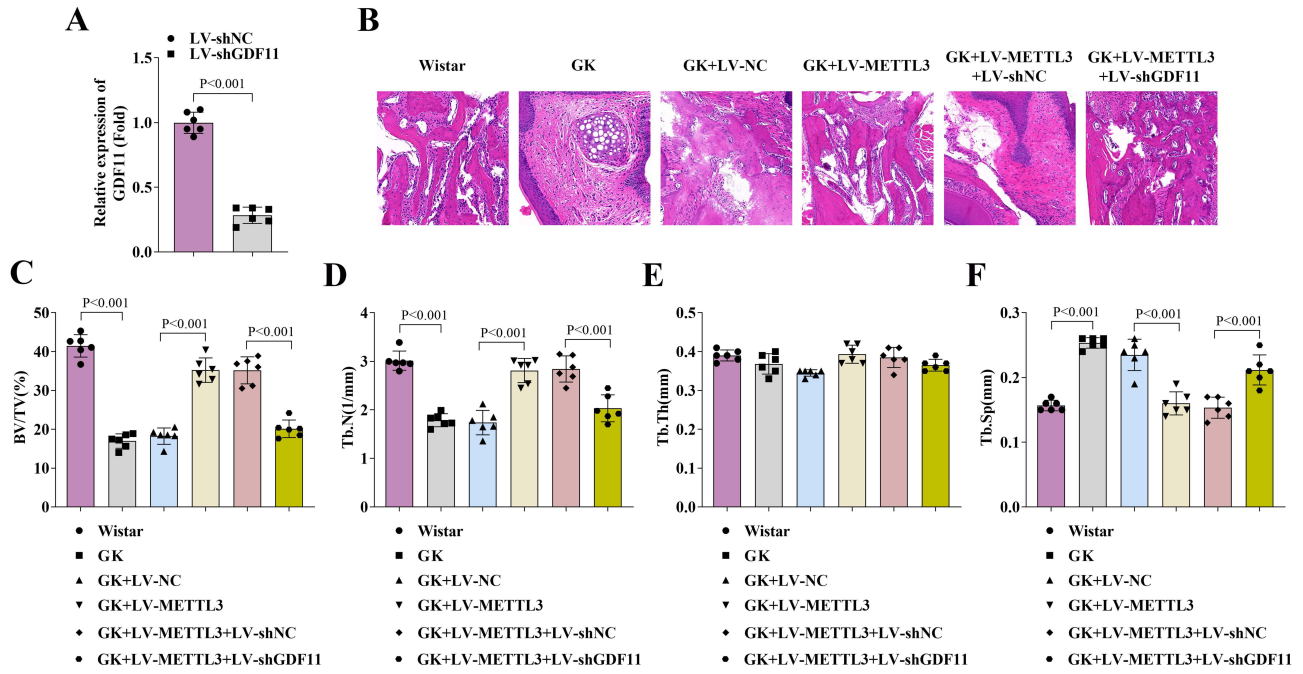

Figure 4 Knockdown of GDF11 partially reverses the tooth extraction socket healing effects of METTL3 overexpression. (**A**) Relative expression of GDF11 in the different groups, n = 6 sockets per group. Data are presented as mean ± SD. $p<0.001$ indicates a statistically significant difference between the LV-shGDF11 group and the LV-shNC group. (**B**) Representative images showing tooth extraction socket healing at the tooth extraction sites in Wistar control, GK, GK+LV-NC, GK+LV-METTL3, GK+LV-METTL3+LV-shNC, and GK+LV-METTL3+LV-shGDF11 groups, n = 6 sockets per group. (**C**–**F**) BV/TV, Tb.N, Tb.Th, and Tb.Sp in the extraction socket 21 days after tooth extraction, n = 6 sockets per group. Data are presented as mean ± SD. $p<0.001$ indicates a statistically significant difference between groups.

overexpression suggests that METTL3 plays a crucial role in the repair process. METTL3-mediated VEGFC m^{6}A modification enhances VEGFR3-mediated lymphangiogenesis to improve wound healing of diabetic foot ulcer.[8] In addition, METTL3-mediated m^{6}A modification also fosters an anti-inflammatory milieu by skewing macrophages toward the pro-reparative M2 phenotype, which is crucial for socket healing.[19,20] Our data is supported by the significant increase in GDF11 expression and the enhanced stability of GDF11 mRNA in the presence of elevated METTL3 levels. The specific m^{6}A modification of GDF11 appears to be critical for this regulatory mechanism, as mutations at this site abolished the beneficial effects of METTL3 overexpression. Our findings also align with previous research indicating that GDF11 is essential for tissue regeneration and repair.[21–23] The partial reversal of tooth extraction socket healing improvements upon GDF11 knockdown further underscores the importance of GDF11 in the METTL3-mediated repair process. This suggests that targeting the METTL3-GDF11 axis could be a promising strategy for enhancing tooth extraction socket healing in diabetic patients.

However, several challenges remain. The precise mechanisms by which m^{6}A modifications influence tooth extraction socket healing in different contexts, such as diabetic and non-diabetic conditions, are still not fully understood. Additionally, the potential side effects and long-term outcomes of modulating METTL3 and GDF11 levels need to be carefully evaluated in future studies. The present study was conducted exclusively in male GK rats, and sex-specific responses to METTL3 modulation remain unexplored. Additionally, the observation period did not extend beyond 8 weeks, precluding conclusions on long-term socket stability or late complications. Future work should include both sexes and prolonged follow-up to validate the translational potential of these findings.

Conclusion

In summary, our data demonstrate that METTL3-mediated m^{6}A modification of GDF11 mRNA accelerates post-extraction socket repair in diabetic rats. While GDF11 is a key downstream effector, we recognise that METTL3 may simultaneously modulate other osteogenic or immune-related transcripts, contributing to the overall healing phenotype. Translation of these findings will require optimisation of lentiviral or nanoparticle delivery systems and careful

evaluation of long-term safety. Moreover, the present study was confined to male rats and followed for only eight weeks; therefore, sex-specific responses and extended outcomes remain to be determined.

Data Sharing Statement

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

This study was approved by the Experimental Animal Welfare Ethics Committee of Qingdao Harwars (Approval Number: IACUC Issue No:AUP-QY-C-S-2025-003).

All animal experiments were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals. Additionally, all methods were carried out in accordance with the ARRIVE guidelines for the reporting of in vivo experiments.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests.

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