Original Article

# Protein Expression after Gingival Injection of mRNA Encoding Platelet-derived Growth Factors-BB in Ligature-induced Periodontitis Model in Rats

**Pimphorn Meekhantong[1,2], Wichaya Wisitrasameewong[1,3,4], Noppadol Sa-Ard-Iam[4], Theeraphat Chanamuangkon[5], Somchai Yodsanga[6], Pimprapa Rerkyen[4], Rangsini Mahanonda[1,3,4]**

[1]Department of Periodontology, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand
[2]School of Dentistry, Mae Fah Luang University, Chiang Rai, Thailand
[3]Center of Excellence in Periodontal Disease and Dental Implant, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand
[4]Immunology Research Center, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand
[5]Biomaterial Testing Center, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand
[6]Department of Oral Pathology, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand

## Abstract

The year 2021 marks the breakthrough of the COVID-19 mRNA vaccine as a new class of medicine. The same fundamentals of the mRNA-based vaccine could facilitate the development of mRNA-based regenerative therapy. Our research group is working on mRNA encoding growth factors for periodontal regeneration in patients with periodontitis. The objective of this study was to investigate protein expression after local administration of mRNA encoding platelet-derived growth factor-BB (PDGF) encapsulated in lipid nanoparticles (PDGF mRNA) in ligature-induced rat periodontitis. 3-0 silk was placed around the maxillary left second molar for two weeks and then removed, while the maxillary right second molar was left non-ligated. A significant bone loss analyzed by a stereomicroscope and micro-computerized tomography and gingival bleeding at the ligature sites were observed as compared to the non-ligature sites. To evaluate transfection and protein translation, different doses of PDGF mRNA including low dose (3 µg), medium dose (10 µg), high dose (30 µg), and DPBS (control) and LNPs alone were injected into rat gingiva at palatal side. The translated PDGF protein production was assessed 24 hours after the injection using enzyme-linked immunosorbent assay (ELISA). High levels of PDGF production were detected at both ligature and non-ligature sites. The mean PDGF levels in mRNA treated groups ranged from 10,912.54±1,893.94 to 51,883.91±7,415.45 pg/mg protein, whereas levels in DPBS control and LNPs alone were negligible. PDGF protein expression showed a trend of dose response but the differences between doses were not significant. Clinical findings at injected sites showed no erythema or swelling. The histological findings showed no evidence of LNPs and other foreign substances of mRNA formulation remaining in the tissues. In conclusion, this study of 24 hour-local delivery of PDGF mRNA-LNPs into rat periodontitis results in highly translated PDGF protein without profound local inflammatory response. However, further studies into the *in vivo* kinetics and therapeutic efficacy of mRNA are required.

**Keywords:** ligature-induced periodontitis, LNPs, mRNA, PDGF, periodontitis

**Received date:** **Revised date:** **Accepted date:**
**Doi:**

**Correspondence to:**
Rangsini Mahanonda, Center of Excellence in Periodontal Disease and Dental Implant, 10th Floor Somdejya 93 Building, Faculty of Dentistry, Chulalongkorn University, 34 Henri-Dunant Road, Wangmai, Patumwan, Bangkok, 10330, Thailand. Tel: 02-218-8849 Fax: 02-218-8851
E-mail: r_mahanonda@yahoo.com

## Introduction

For the past few decades, intensive research based on tissue engineering strategies for periodontal tissue regeneration has been the use of stem cells, scaffold, and signaling molecules. Many growth factors such as platelet-derived growth factors-BB (PDGF), insulin-like growth factors, fibroblast growth factors, bone-morphogenetic protein (BMP) have shown potential to regenerate the lost periodontal tissues.[1] Recombinant human PDGF-BB (rhPDGF), GEM 21S® (Osteohealth, USA), has become the first growth factor product, which was clinically approved by the U.S. FDA in 2005 for periodontal regeneration. However, one of its limitations in clinical application is the relatively short half-life of growth factors *in vivo*, which typically range from several hours to days. As a result, supraphysiologic doses or several administrations are required, and such high doses of growth factors may cause undesirable side effects and increase the cost of therapy.[2] Gene therapy is considered as an alternative approach to address the drawbacks of protein delivery. The gene therapy involves a delivery of DNA or mRNA encoding protein of interest into the cells, thus allowing cell transfection and protein translation to occur. Hence, one's own body makes the desired protein by themselves.[3]

In 2021, mRNA-based technology emerged in the field of medicines by the first mRNA vaccine against SARS-CoV-2, which was approved by the U.S. FDA.[4] This mRNA vaccine uses nucleoside-modified mRNA encoding, a spike protein antigen, which is encapsulated with lipid nanoparticles (LNPs). It is known that nucleoside modified mRNA translated more protein than unmodified mRNA by suppressing an inflammatory response, which can interfere with protein translation.[5] Encapsulation of mRNA with LNPs can protect mRNA from degradation by extracellular ribonucleases mRNA and make mRNA more stable.[6] Hence, mRNA-LNPs technology has been recognized as the most advanced platform in medicine. Now more than a billion doses of mRNA vaccine have been used around the world showing that the mRNA vaccine is both safe and effective.[7] Likewise, the same mRNA technology platform could be applied for mRNA-based therapeutics.

In the field of mRNA-based regenerative medicine, Zangi *et al.,* (2013) showed that the use of modified mRNA encoding human vascular endothelial growth factor-A (VEGF-A mRNA) in a mouse myocardial infarction model could enhance the formation of new blood vessels in infarction area and the survival rate increased as compared to controls (no mRNA administration).[8] The phase 2 study showed safety and improved heart function after direct injections of VEGF-A mRNA into the hearts of 11 patients with coronary diseases during open heart surgery, suggesting a promising clinical outcome of VEGF-A mRNA treatment for heart tissue regeneration.[9] Currently, the phase 3 clinical study of VEGF-A mRNA in a larger number of heart failure patients are ongoing, and the results are pending till next year.[10]

Our research group has studied the potential use of mRNA encoding growth factors for periodontal regeneration. High protein expression was demonstrated after 24 hour-transfection *in vitro* of primary human periodontal ligament cells and primary human gingival fibroblasts with modified mRNA encoding PDGF.[11] Furthermore, we demonstrated *in vivo* that direct injection of modified PDGF mRNA in healthy rat gingiva induced high protein translation.[12] Therefore, the present study investigated protein translation following gingival injections of PDGF mRNA in rat periodontitis. A ligature-induced periodontitis in rats was selected as a study model since it mimics the pathogenesis of periodontitis in humans caused by dental plaque accumulation around the ligature.[13] Different mRNA doses were also tested. Findings from this study will provide information regarding the use of ligature-induced periodontitis in rats for further investigation of PDGF mRNA therapeutic efficacy for periodontal regeneration.

## Materials and methods

Construction of N1-methylpseudouridine - modified mRNA encoding PDGF

The nucleotide sequence of human PDGF was designed by Professor Rangsini Mahanonda and Professor Sathit Pichyangkul, and the N1-methylpseudouridine - modified mRNA encoding PDGF was synthesized by Dr. Norbert Pardi (University of Pennsylvania, USA).[14]

Ligature-induced experimental periodontitis in rats

Animal care and experimental procedures were approved by the Ethics committee at Faculty of Tropical

Medicine-Institute Animal Care and Use Committee at Mahidol University (Certificated no. FTM-IACUC 012/2019). Sprague-Dawley male rats (six weeks old) were purchased from Nomura Siam International Co.,Ltd. (Bangkok, Thailand) and adopted in individually ventilated cages with a 12-hour light/dark cycle for a week before the beginning of the experiment. Figure 1A demonstrated experimental design of ligature-induced rat periodontitis. Rats were anaesthetized by intraperitoneal injection with Zoletil (40 mg/kg) and Xylazine (5 mg/kg). Induction of experimental periodontitis in rat, silk ligatures (3-0 silk threads, Johnson & Johnson, New Brunswick, NJ, USA) were placed on the maxillary left second molar for two weeks (Fig. 1B). Suture was tied firmly with a double-knot on the buccal side of the maxillary left second molar. The maxillary right second molar was left non-ligature (Fig. 1B). The animal was fed with regular diet, received postoperative care, and the ligature was checked at one week post-ligation.

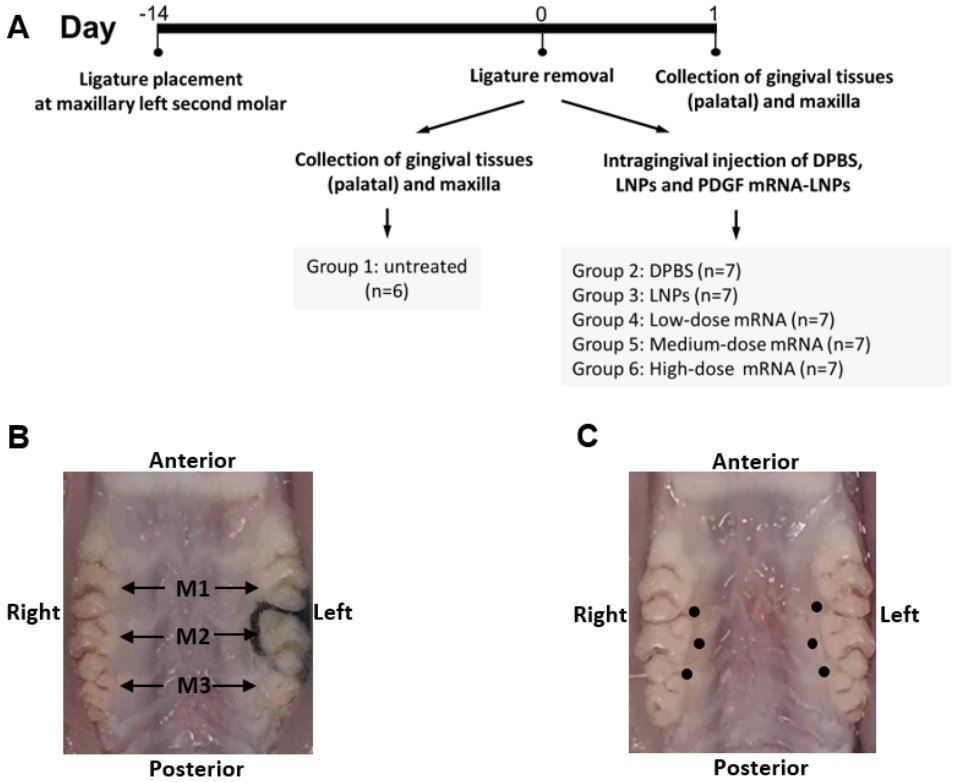


***Figure 1*** *Overview of the experimental design. (A) Diagram of ligature-induced rat periodontitis and the intra gingival delivery of DPBS, LNPs, and PDGF mRNA-LNPs. (B) In each rat, ligature was placed around the maxillary left second molar while the maxillary right second molar was not ligated. (C) Black dots indicate the injection sites. (DPBS = Dulbecco's phosphate-buffered saline, LNPs = lipid nanoparticles, PDGF mRNA-LNPs = N1-methylpseudouridine - modified mRNA encoding platelet derived growth factor-BB encapsulated with LNPs).*

Administration of DPBS, LNPs and N1-methylpseudouridine mRNA encoding PDGF in LNPs

All ligatures were removed after two weeks of ligation The animals were randomized into six groups (six to seven animals per group) as demonstrated in Figure 1A. The first group was immediately sacrificed and served as the untreated group. Groups 2 to 6 received different substances by intra gingival injections. The injection was performed at six sites (Fig. 1C) with the volume of 6 µl solution per site, which contained DPBS (group2, control); LNPs only (group3); 3 µg PDGF mRNA (group4, low-dose mRNA); 10 µg PDGF mRNA (group5, medium-dose mRNA); and 30 µg PDGF mRNA (group6, high-dose mRNA). A total volume of 36 µl solution was given to each rat. All animals (group 2-6) were sacrificed 24 hours after administration.

In each group, four rats were harvested for maxillae. In each maxilla, gingival tissues (palatal side) were used for measurement of protein production, while the remaining

maxillae were used for alveolar bone measurement. The other two to three rats in each group were used for micro-computerized tomography (micro-CT) imaging and subsequently for histological analysis.

Measurement of alveolar bone level

Rat maxillae were dissected and defleshed in 5% sodium hypochlorite for seven days. Pictures of these samples were taken under stereomicroscope (Olympus SZ61; Olympus Corporation, Tokyo, Japan) and analyzed alveolar bone level by ImageJ 1.52a software program (National Institutes of Health, USA). Two to three maxillae per group were scanned under a micro-CT system (Micro-CT µ35 scanco; SCANCO medical, Brüttisellen, Switzerland) and generated 3D images of the maxillae. All images of the maxillae were analyzed at the buccal and palatal sites. To identify alveolar bone loss, the mean distances from cementoenamel junction (CEJ) to alveolar bone crest (ABC) were measured at five sites on each surface (distobuccal or distopalatal line angle of maxillary first molar, mesiobuccal or mesiopalatal line angle, mid-buccal or mid-palatal, distobuccal or distopalatal line angle of maxillary second molar, mesiobuccal or mesiopalatal line angle of maxillary third molar).

Measurement of protein production

Gingival tissues at the palatal side were collected with a sulcular incision at the mesiopalatal line angle of maxillary first molar to distopalatal line angle of maxillary third molar and a horizontal incision was made approximately 2-3 mm below the gingival margin. The collected gingiva was weighed and homogenized in RIPA (extract protein) with protease inhibitor cocktail (Sigma, St. Louis, MO, USA). The homogenates were then centrifuged at 16,000 rpm for 15 minutes at 4°C. The amount of total proteins was measured by a BCA protein assay kit (PierceTM BCA Protein Assay; Thermo Scientific, Co., Ltd., Rockford, IL USA). The levels of PDGF protein, IL-1$\beta$ and TNF-$\alpha$ were measured using enzyme-linked immunosorbent assay (ELISA) kits (Quantikine® ELISA; R&D System, Inc., Minneapolis, MN, USA). The sensitivity of the IL-1$\beta$ and TNF-$\alpha$ was < 5 pg/ml, according to the manufacturer.

Histological evaluation

After micro-CT scanning, the maxillae were decalcified in 10 % ethylenediaminetetraacetic acid solution at 4°C for two weeks. Decalcified maxillae were dehydrated and embedded in paraffin. The serial section of specimens was performed in 7 µm thickness in mesio-distal direction and stained with hematoxylin and eosin (H&E) to analyze inflammatory cell infiltration.

Statistical analysis

All data were analyzed by the statistical software SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). Data between non-ligated site and ligated site were compared by Paired *T*- test. Differences among groups were analyzed by ANOVA followed by multiple comparisons with Bonferroni's post hoc test. The statistical significance was considered as *P*-value < 0.05.

## Results

### Ligature-induced periodontitis model in rats

In group 1, after two weeks of ligature placement, the mean alveolar bone loss of the ligature site was 0.7 ± 0.04 mm (0.63 ± 0.05 mm on buccal sides and 0.78 ± 0.07 mm on palatal sides). Whereas the mean alveolar bone loss of non-ligature sites was 0.5 ± 0.05 mm (0.35 ± 0.02 mm on buccal sides a nd 0.66 ± 0.08 mm on palatal sides) (Fig. 2A - 2C). There was significantly more bone loss at the ligature sites than at the non-ligature sites, indicating an established periodontitis model with periodontal bone loss in rats.

In group 1, both non-ligature and ligature sites showed low levels of inflammatory cytokine IL-1$\beta$ < 50 pg/mg protein, with no significant differences between the two sites (Fig. 2D). TNF-$\alpha$ was not detected.

H&E stained sections of the ligature sites showed ulcerated epithelium, crestal bone loss and the presence of inflammatory infiltrates (Fig. 3E,3F), while non-ligature sites showed intact sulcular epithelium and some cellular infiltrates (Fig. 3B,3C). These histological findings corresponded to the 3D micro-CT imaging (Fig. 3D, 3A).

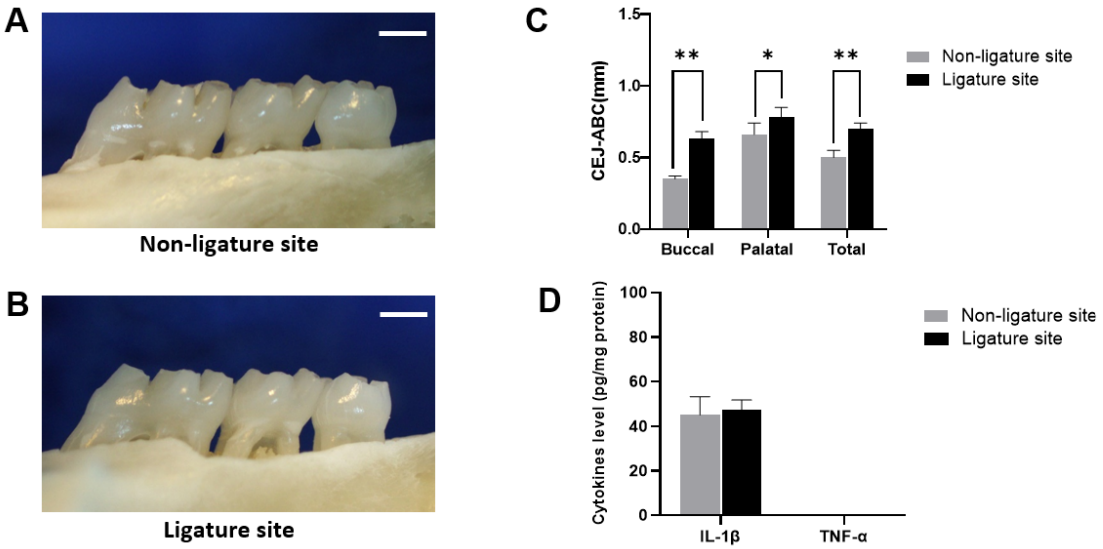


*Figure 2* *Bone loss and cytokine production after 14 days of non-ligature and ligature sites in group 1. Representative stereomicroscope images from the buccal surfaces of (A) non-ligature, and (B) ligature sites (20x magnification; scale bar = 1 mm) (n=4). (C) The distance (mm) was from cementoenamel junction (CEJ) to alveolar bone crest (ABC), the linear measurement under stereo-microscope. Data shown are mean ± SE of the CEJ-ABC distance from the buccal side, palatal side, and total (the sum of buccal and palatal sides) (n = 4; *p < 0.05; ** p < 0.001, compared between non-ligature and ligature sites; Paired T- test) and (D) inflammatory cytokine levels of IL-1β and TNF-α in gingival tissues (palatal site). Data shown are mean ± SE (n = 4). (IL-1β = interleukin-1 beta, TNF-α = Tumor necrosis factor-alpha)*

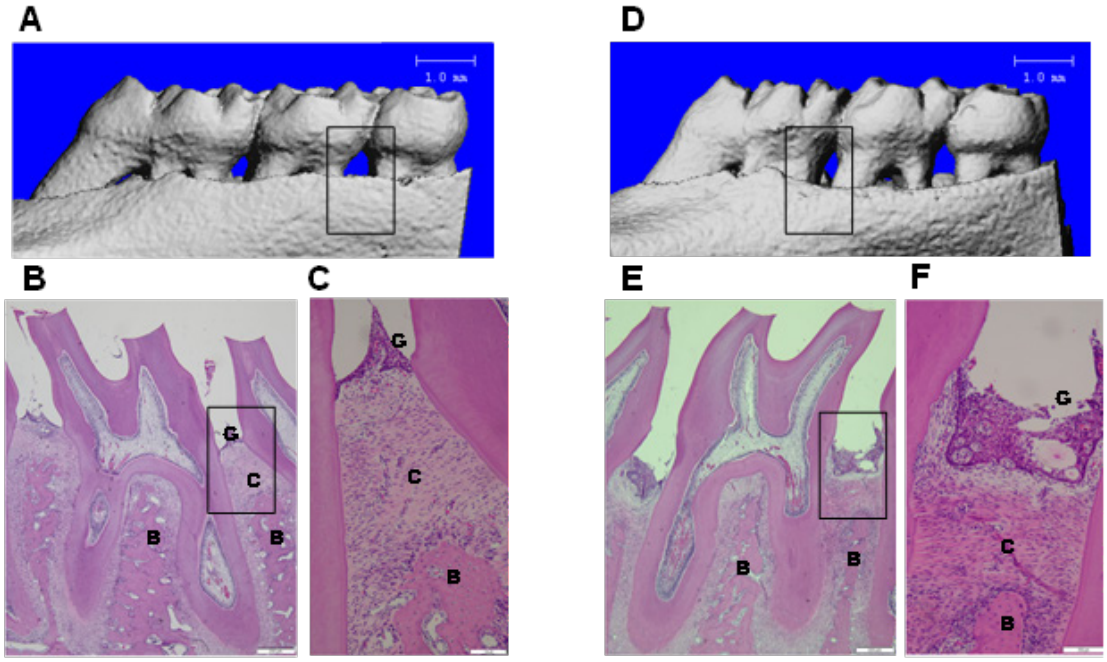


*Figure 3* *Representative of micro-CT images and histological findings after 14 days of (A) non-ligature and (D) ligature sites in group 1. The corresponding H&E-stained sections of (B&C) showed intact sulcular epithelium and some inflammatory cell infiltrates whereas (E&F) showed ulcerated epithelium and the presence of inflammatory cell infiltrates in the ligature site. (B&E; original magnification x4; C&F; original magnification x20; B= alveolar bone; G= gingival epithelium; C= connective tissue)*

**Expression of PDGF and inflammatory cytokines after local delivery of PDGF mRNA-LNPs in rat periodontitis model**

After 24 hours of gingival injection of PDGF mRNA, clinical findings at the injected sites showed no erythema or swelling. The expression levels of translated PDGF protein tended to increase as mRNA dose increased at both non-ligature and ligature sites (group 4, 5, 6) (Fig. 4A). At each mRNA dose, there was no statistically significant difference in protein production between non-ligature and ligature sites. Negligible amount of PDGF protein was detected in DPBS control (group 2, ranging from 19.26 ± 2.01 to 24.13 ± 3.93 pg/mg protein) and LNPs (group 3, ranging from 18.79 ± 1.83 to 21.96 ± 2.3 pg/mg protein) (Fig. 4A). At the non-ligature sites, the mean PDGF protein was 51,883.91 ± 7,415.45 pg/mg protein at the high-dose mRNA; 24,666.22 ± 5,782.93 pg/mg protein at the medium-dose mRNA; and 10,912.54 ± 1.893.94 pg/mg protein at the low-dose mRNA group. It was found that the medium-dose mRNA and high-dose mRNA groups resulted in significantly higher protein production than the control and LNPs groups. In addition, the translated protein in the high-dose mRNA group was significantly higher than in the low-dose mRNA and medium-dose mRNA groups. At the ligature sites, the mean PDGF protein was 48,012.66 ± 16,063.13 pg/mg protein at the high-dose mRNA; 41,134.63 ± 10,430.55 pg/mg protein at the medium-dose mRNA; and 15,918.79 ± 7,681.11 pg/mg protein at the low-dose mRNA. It was found that the mean protein levels were significantly higher only in the high-dose mRNA-LNPs group than the control and LNPs groups.

In addition to PDGF protein production, the levels of inflammatory cytokines including IL-1β and TNF-α were assessed after injection of PDGF mRNA. TNF-α production was undetected in all groups, whereas IL-1β production was observed with the mean ranging from 72.08±13.24 to 1,458.44 ±180.51 pg/mg protein. The IL-1B level was higher in LNPs and high dose of mRNA groups compared to the other groups, however the difference was not statistically significant. (Fig. 4B).

The H&E staining showed no evidence of LNPs and other foreign substances of mRNA formulation remaining in the tissues. There was no significant inflammatory infiltration in any of the animals that received PDGF mRNA (data not shown).

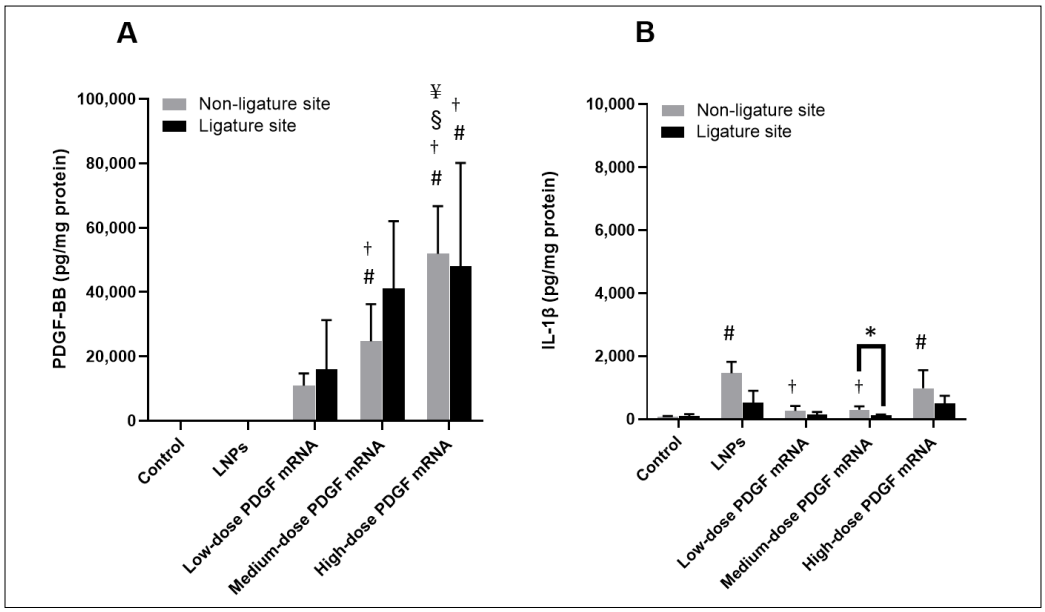


***Figure 4*** *Production of (A) PDGF protein and (B) IL-1β in palatal tissues after gingival injection with DPBS control, LNPs and PDGF mRNA (low, medium, and high doses) in non-ligature and ligature sites. Data shown are mean ± SE (*p<0.05, compared between non-ligature and ligature sites; #p<0.05, compared to control group; †p<0.05, compared to LNPs group; §p<0.05, compared to low-dose mRNA group; ¥p<0.05, compared to medium-dose mRNA group; one-way ANOVA and Bonferroni's post hoc tests), (DPBS = Dulbecco's phosphate-buffered saline, LNPs = lipid nanoparticles, PDGF mRNA = N1-methylpseudouridine - modified mRNA encoding platelet derived growth factor-BB encapsulated LNPs)*

## Discussion

This study is the first study to explore the potential application of mRNA encoding PDGF-BB for periodontal regeneration in periodontitis. Local delivery of PDGF-BB mRNA in LNPs in ligature-induced rat periodontitis demonstrated a high production of the PDGF-BB protein. Although the translated protein showed a dose response trend, the differences in protein levels among different mRNA doses were not statistically significant.

The ligature model was used to establish periodontitis in the present study since it is a well-known approach to induce periodontitis and has been used in many relevant studies for testing efficacy of biological reagents.[15] This model is reported to be similar to human periodontitis in various aspects, as the alveolar bone resorption depends on bacterial plaque and inflammation of gingival tissue. Numerous studies of ligature-induced periodontitis in rodents showed alveolar bone loss, which is a hallmark of periodontitis.[13,15,16] In this study, the ligature-induced periodontitis model was successfully established in rats by ligation, as the alveolar bone loss at ligature sites was significantly greater than at non-ligature sites on both buccal and palatal surfaces. Our results supported previous studies that used a similar technique with 3-0 silk for 2-week ligation.[17] However, the level of inflammatory cytokine and the presence of inflammatory infiltrates were not different between the ligature site and the non-ligature site. This could possibly be explained by the timing of tissue collection for cytokine and histological analysis. Our study was conducted on day 14 after ligation, which was later than some other previous studies.[18-20] Alveolar bone loss with intense infiltration of inflammatory cells was observed at days 7-9 following ligature placement and then markedly declined.[18,19] IL-1$\beta$ and TNF-$\alpha$ gene expression was also significantly elevated as early as days 1-3.[18,20] Those studies used real-time PCR to measure mRNA expression, whereas our study used ELISA, a reliable and appropriate method for investigating the protein levels of secreting cytokines. In addition to observation period, differences in host genetic variations on immune responses and plaque accumulation may contribute to variation in inflammation.

In a previous *in vivo* study of kinetic protein expression following local delivery of pseudouridine-modified PDGF mRNA in a healthy rat, the peak of PDGF protein translation was observed in gingival tissues 24 hours after intra gingival injection.[12] Therefore, the time frame used for protein analyses in this study was 24 hours.

The mRNA formulation used in this study was N1-methylpseudouridine-modified mRNA encapsulated with LNPs. In comparison to our previous study with pseudouridine-modified mRNA, N1-methylpseudouridine-modified mRNA encapsulated with LNPs resulted in higher protein translation and expression at the same time point (24 hours following intra gingival injection).[12] This could be explained by the use of the modified nucleobase N1-methylpseudouridine that has been shown to effectively decrease intracellular innate immune signals and, thus, improve mRNA stability.[21] In addition to the mRNA based modification, LNPs were employed to protect mRNA from RNase degradation and promote intracellular entry.[22] Because of their ease of manufacture and ability to improve mRNA translational capacity, LNPs are widely used as a carrier for mRNA, for example, in mRNA COVID-19 vaccine. The structure of LNPs consist of phospholipids, cholesterol, ionizable cationic lipids and PEGylated lipids for support, stabilization, complexation of negatively charged mRNA molecules, facilitating endosomal escape, and reducing nonspecific endocytosis by host immune cells, respectively.[23-25] However, previous pre-clinical studies reported that cationic lipid component in LNPs could activate an inflammatory response via NF-$\kappa$B activation, and the production of TNF-$\alpha$, IL-1$\beta$, IL-6 and IFN-$\gamma$.[26,27] In this study, we found that LNPs and high-dose mRNA groups had higher level of IL-1$\beta$ in compared to other groups, however, we did not observed clinical swelling or erythema at the injected gingiva. Although, LNPs-induced inflammatory response could serve as an effective adjuvant for an mRNA-LNPs vaccine, this inflammation may be unfavorable for

tissue regeneration. Therefore, development of new types of noninflammatory delivery molecules that protect therapeutic mRNA from degradationand facilitate its cellular uptake would be required to address the issue of LNP-induced immune system activation.

Safety is a major concern when using mRNA as a therapeutic option in patients with periodontitis. The mRNA dosage used in our experiment ranges from 3 - 30 µg PDGF-BB mRNA/animal, which is considered lower than the previous study by Zangi *et al.* (2013). They injected 200 µg VEGF mRNA/animal into the myocardium of mouse myocardial infarction model. Their results showed improved heart function, no adverse effects and enhanced long term survival (1 year).[8] Of importance, it is becoming clear that COVID-19 nucleoside- modified mRNA-LNP vaccines have shown a strong safety and efficacy profile (>90%)[28] and to date, 12.68 billion doses of these vaccines have been used around the world.[29] The success of an mRNA vaccine is likely to facilitate the development of other mRNA-based therapeutic products. However, a future long-term study investigating safety and therapeutic efficacy of PDGF-BB mRNA in small and large animals is required.

## Conclusion

PDGF expression was detected in gingival tissues in a periodontitis model following an intra gingival injection of PDGF mRNA-LNPs without profound local inflammatory response. A further study is required to evaluate the therapeutic effect of PDGF-BB mRNA.

## Acknowledgement

This study was supported by Thailand Science Research and Innovation (DIG6280002). We would like to thank Dr. Jetsumon Prachumsri and Dr. Niwat Kangwarangsan for their advice on the animal procedures, and also Assist. Prof. Soranun Chantarangsu for statistical analysis suggestions.

## References

1. Bartold PM, McCulloch CA, Narayanan AS, Pitaru S. Tissue engineering: a new paradigm for periodontal regeneration based on molecular and cell biology. ***Periodontol 2000*** 2000;24:253-69.

2. Carragee EJ, Hurwitz EL, Weiner BK. A critical review of recombinant human bone morphogenetic protein-2 trials in spinal surgery: emerging safety concerns and lessons learned. ***Spine J*** 2011;11(6):471-91.

3. Magadum A, Kaur K, Zangi L. mRNA-Based Protein Replacement Therapy for the Heart. ***Mol Ther*** 2019;27(4):785-93.

4. Baden LR, El Sahly HM, Essink B, Kotloff K, Frey S, Novak R, *et al.* Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. N ***Engl J Med*** 2021;384(5):403-16.

5. Karikó K, Muramatsu H, Welsh FA, Ludwig J, Kato H, Akira S, *et al.* Incorporation of pseudouridine into mRNA yields superior nonimmunogenic vectors with increased translational capacity and biological stability. ***Mol Ther*** 2008;16(11):1833-40.

6. Hou X, Zaks T, Langer R, Dong Y. Lipid nanoparticles for mRNA delivery. ***Nature Reviews Materials*** 2021;6(12):1078-94.

7. Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines - a new era in vaccinology. ***Nat Rev Drug Discov*** 2018;17(4):261-79.

8. Zangi L, Lui KO, von Gise A, Ma Q, Ebina W, Ptaszek LM, *et al.* Modified mRNA directs the fate of heart progenitor cells and induces vascular regeneration after myocardial infarction. ***Nat Biotechnol*** 2013;31(10):898-907.

9. Anttila V, Saraste A, Knuuti J, Jaakkola P, Hedman M, Svedlund S, *et al.* Synthetic mRNA Encoding VEGF-A in Patients Undergoing Coronary Artery Bypass Grafting: Design of a Phase 2a Clinical Trial. ***Mol Ther Methods Clin Dev*** 2020;18:464-72.

10. Collén A, Bergenhem N, Carlsson L, Chien KR, Hoge S, Gan LM, *et al.* VEGFA mRNA for regenerative treatment of heart failure. ***Nat Rev Drug Discov*** 2022;21(1):79-80.

11. Surisaeng T, Mahanonda R, Sa-Ard-Iam N, Rerkyen P, Chanamuangkon T, Chanpaiboon C, editors. The use of modified mRNA encoding platelet-derived growth factor-BB as an innovation in periodontal regeneration. Proceedings of RSU International Research Conference 2020; 2020; Pathum Thani, Thailand.

12. Bhongsatiern P, Mahanonda R, Sa-Ard-Iam N, Chanamuangkon T, Wisitrasameewong W, editors. Protein expression after delivery of mRNA encoding PDGF-BB into rat gingiva: A Pilot Study. Proceedings of the 15th RSU National Graduate Research Conference; 2020; Pathum Thani, Thailand. 2020.

13. Graves DT, Fine D, Teng YT, Van Dyke TE, Hajishengallis G. The use of rodent models to investigate host-bacteria interactions related to periodontal diseases. ***J Clin Periodontol*** 2008;35(2):89-105.

14. Pardi N, Tuyishime S, Muramatsu H, Kariko K, Mui BL, Tam YK, *et al.* Expression kinetics of nucleoside-modified mRNA delivered in lipid nanoparticles to mice by various routes. ***J Control Release*** 2015; 217:345-51.

15. Lin P, Niimi H, Ohsugi Y, Tsuchiya Y, Shimohira T, Komatsu K, *et al.* Application of Ligature-Induced Periodontitis in Mice to Explore the Molecular Mechanism of Periodontal Disease. ***Int J Mol Sci*** 2021;22(16).

16. Graves DT, Li J, Cochran DL. Inflammation and uncoupling as mechanisms of periodontal bone loss. ***J Dent Res*** 2011;90(2):143-53.
17. Liu YF, Wu LA, Wang J, Wen LY, Wang XJ. Micro-computerized tomography analysis of alveolar bone loss in ligature- and nicotine-induced experimental periodontitis in rats. ***J Periodontal Res*** 2010; 45(6):714-9.
18. Marchesan J, Girnary MS, Jing L, Miao MZ, Zhang S, Sun L, *et al.* An experimental murine model to study periodontitis. ***Nat Protoc*** 2018;13(10):2247-67.
19. Wu YH, Taya Y, Kuraji R, Ito H, Soeno Y, Numabe Y. Dynamic microstructural changes in alveolar bone in ligature-induced experimental periodontitis. ***Odontology*** 2020;108(3):339-49.
20. de Molon RS, Park CH, Jin Q, Sugai J, Cirelli JA. Characterization of ligature-induced experimental periodontitis. ***Microsc Res Tech*** 2018;81(12):1412-21.
21. Andries O, McCafferty S, De Smedt SC, Weiss R, Sanders NN, Kitada T. N(1)-methylpseudouridine-incorporated mRNA outperforms pseudouridine-incorporated mRNA by providing enhanced protein expression and reduced immunogenicity in mammalian cell lines and mice. ***J Control Release*** 2015;217:337-44.
22. Kowalski PS, Rudra A, Miao L, Anderson DG. Delivering the Messenger: Advances in Technologies for Therapeutic mRNA Delivery. ***Mol Ther*** 2019;27(4):710-28.
23. Kauffman KJ, Dorkin JR, Yang JH, Heartlein MW, DeRosa F, Mir FF, *et al.* Optimization of Lipid Nanoparticle Formulations for mRNA Delivery in Vivo with Fractional Factorial and Definitive Screening Designs. ***Nano Lett*** 2015;15(11):7300-6.
24. Semple SC, Leone R, Barbosa CJ, Tam YK, Lin PJC. Lipid Nanoparticle Delivery Systems to Enable mRNA-Based Therapeutics. ***Pharmaceutics*** 2022;14(2).
25. Kulkarni JA, Cullis PR, van der Meel R. Lipid Nanoparticles Enabling Gene Therapies: From Concepts to Clinical Utility. ***Nucleic Acid Ther*** 2018;28(3):146-57.
26. Ndeupen S, Qin Z, Jacobsen S, Bouteau A, Estanbouli H, Igyártó BZ. The mRNA-LNP platform's lipid nanoparticle component used in preclinical vaccine studies is highly inflammatory. ***iScience.*** 2021; 24(12):103479.
27. Lonez C, Bessodes M, Scherman D, Vandenbranden M, Escriou V, Ruysschaert JM. Cationic lipid nanocarriers activate Toll-like receptor 2 and NLRP3 inflammasome pathways. ***Nanomedicine*** 2014;10(4):775-82.
28. Hogan MJ, Pardi N. mRNA Vaccines in the COVID-19 Pandemic and Beyond. ***Annu Rev Med*** 2022;73:17-39.
29. Ourworldindata.org [Internet]. Oxford: Coronavirus (COVID-19) vaccinations; 2021 [updated 2022 Sep 18; cited 2022 Sep 18]. Available from: https://ourworldindata.org/covid-vaccinations