Original Articles

In vivo Protein Expression after Delivery of Modified mRNA Encoding Bone Morphogenetic Protein - 2 into Rat Gingiva Using Different Delivery Systems: A Pilot Study

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Abstract

In vitro transcribed (IVT) nucleoside-modified messenger ribonucleic acid (mRNA) has emerged as a novel platform in regenerative medicine. Given the potential of a mRNA platform to encode any protein of interest and produce it directly *in vivo*, mRNA encoding growth factor could be a promising alternative to recombinant protein in promoting periodontal tissue and bone regeneration. Bone morphogenetic protein-2 (BMP-2) is one of most extensively studied growth factors due to its osteogenic potential. To date, an *in vivo* study of mRNA encoding BMP-2 therapy in the field of periodontology is scarce. In addition, a proper mRNA delivery system has yet to be identified. Therefore, this study aimed to examine the effect of N1-methylpseudouridine-modified mRNA encoding BMP-2 (m1Ψ-BMP-2 mRNA) with different delivery systems on the level of BMP-2 protein and inflammatory response at local tissue upon intragingival injection. Twelve Sprague-Dawley rats were randomly assigned into four groups injected with m1Ψ-BMP-2 mRNA in Dulbecco's phosphate-buffered saline (dPBS), sucrose citrate buffer, Lipofectamine® 2000, or dPBS alone without mRNA as a control at palatal gingiva. Gingival tissues were collected at 24 hours for analysis of BMP-2 protein and pro-inflammatory cytokines (interleukin-1β, interleukin-6, and tumor necrosis factor-α) using enzyme-linked immunosorbent assay. The highest BMP-2 protein expression in gingival tissue was observed in a rat injected with m1Ψ-BMP-2 mRNA in Lipofectamine® 2000. The amount of interleukin-1β in all the groups was less than 100 pg/mg of protein, while interleukin-6 and tumor necrosis factor-α were undetected. The findings from this *in vivo* study demonstrated that intragingival injection of m1Ψ-BMP-2 mRNA in Lipofectamine® 2000 resulted in increased BMP-2 production with minimal local immune responses. Our preliminary data suggested that intragingival injection of m1Ψ-BMP-2 mRNA was able to promote BMP-2 protein expression in gingival tissues. Nucleoside-modified mRNA could be a potential therapeutics platform for periodontal regeneration; however, further studies are required.

Keywords: Bone morphogenetic protein-2, Delivery system, mRNA, Periodontal regeneration

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Introduction

Periodontitis is a chronic inflammatory disease resulting from the host response against bacterial plaque and its components, which can be modified by genetic and environmental factors. The disease is characterized by destruction of periodontium including gingiva, periodontal ligament, cementum and alveolar bone. Alveolar bone resorption, a hallmark of periodontitis, can eventually lead to tooth loss in severe cases.[1] The aims of periodontal treatment are not only to eliminate inflammation and infection, but also to regenerate structures and function of the destructed periodontal tissues. Periodontal regenerative therapy is, therefore, the treatment of choice whenever possible. However, the treatment outcome of current approaches including guided tissue regeneration (GTR) and guided bone regeneration (GBR) with available biomaterials is limited.[2,3] Studies have identified various factors affecting the treatment outcomes. Although satisfied results have been clinically demonstrated in some cases, complete periodontal regeneration is rarely obtained histologically.[2] The use of biological proteins, such as enamel matrix derivative (EMD), recombinant human platelet-derived growth factor (rhPDGF) and recombinant human bone morphogenetic protein (rhBMP), have been investigated to achieve periodontal tissue regeneration.[4]

Bone morphogenetic protein-2 (BMP-2) has gained interest in the field of periodontology due to its osteogenic potential. It was demonstrated that BMP-2 has an ability to stimulate intramembranous bone formation without an endochondral intermediate[5], and also cementogenesis.[6] The stimulatory effect of BMP-2 on periodontal ligament (PDL) formation is, however, controversial.[5,7] Nevertheless, early studies on animals have suggested the potential of BMPs in enhancing periodontal regeneration.[7] Clinically, the therapeutic use of recombinant human BMP-2 (rhBMP-2) was approved by the U.S. Food and Drug Administration (FDA) and the products are commercially available. However, clinical outcomes regarding the use of rhBMP-2 are inconsistent. This is probably due to its short half-life *in vivo.* In addition, similar to other therapeutic proteins, information on dosage and long-term effects of rhBMP-2 is still lacking.[4,8]

Gene therapy is, therefore, in the spotlight as an alternative to therapeutic proteins.[9] Messenger ribonucleic acid (mRNA)-based therapy, in comparison to deoxyribonucleic acid (DNA)-based therapy, overcomes the need for nuclear localization and does not integrate into the host chromosomes. Additionally, the use of mRNA is safer since mRNA translation is transient and sustained for a short period of time before completely degraded through physiologic pathways.[10] The major limitations of mRNA in aspects of immunogenicity and instability were overcome by incorporating modified nucleosides in mRNA and using appropriate delivery systems.[11] The incorporation of N1-methylpseudouridine ($m1\Psi$) outperformed other modifications in terms of high translational capacity and reduced innate immune responses among the other modifications studied.[12] The use of lipid encapsulation as a carrier could improve mRNA stability by ensuring protection from extracellular RNase degradation while also facilitating the cellular uptake of mRNA and subsequently endosomal escape.[13] In this regard, lipids and lipid-like materials, such as lipofectamine, have been used for mRNA delivery both *in vitro* and *in vivo* studies. They could provide successful mRNA transfection and protein production in multiple cell types, including fibroblasts and other periosteal cells.[14,15] Besides lipids, sucrose citrate buffer has also been investigated and satisfying results have been reported.[16-18] Promising results of mRNA technology from multiple preclinical studies in the field of tissue regeneration[19-24], mRNA encoding vascular endothelial growth factor A (VEGF-A) have now been under clinical trials, aiming to develop regenerative therapies for the treatment of cardiometabolic diseases.[25,26] Nevertheless, the use of nucleoside modified mRNA has never been investigated in the field of periodontal tissue regeneration.

Taking the potential of mRNA-based therapy into account, a mRNA encoding specific growth factor could be a promising alternative for regenerative periodontal therapy. Our research team has developed nucleoside-modified mRNA encoding BMP-2 and successfully demonstrated its transfection and expression into target cells *in vitro*. It was hypothesized that this nucleoside–modified mRNA encoding BMP-2 could be delivered into gingiva and translated into protein *in vivo*. Therefore, in this study, it was proposed

to investigate the effect of local delivery of m1Ψ-BMP-2 mRNA on BMP-2 protein expression and inflammatory response in gingiva. Two different delivery systems including sucrose citrate buffer and Lipofectamine® 2000 were tested.

Materials and methods

Construction of modified mRNA encoding BMP-2

Nucleotide sequences of human BMP-2 were designed by Prof. Rangsini Mahanonda and her team. In collaboration, the synthesis of N1-methylpseudouridine modified mRNA was kindly provided by Dr. Norbert Pardi from University of Pennsylvania.[27]

Animals

Animal care and use protocol and ethical approval were obtained from the Institute of Animal Care and Use Committee of the Faculty of Tropical Medicine, Mahidol University (FTM-IACUC) and the Ethics committee of the Faculty of Dentistry, Chulalongkorn University. Wild-type Sprague-Dawley male rats, aged 6-weeks-old, were purchased from Nomura Siam International Co., Ltd. (Bangkok, Thailand) and were adopted in conventional animal housing with a 12-h light-dark cycle at constant temperature for one week before beginning the experiment. The number of rats per group was three (based on a previous study).[28]

Delivery of mRNA encoding BMP-2 with sucrose citrate buffer or Lipofectamine® 2000 into rat gingiva

Animals were anaesthetized with sodium pentobarbital (50 mg/kg body weight). Animals were randomly assigned into four groups (N = 3 rats/group) using different delivery systems: 1) m1Ψ-BMP-2 mRNA in Dulbecco's phosphate-buffered saline (dPBS) 2) m1Ψ-BMP-2 mRNA in sucrose citrate buffer 3) m1Ψ-BMP-2 mRNA complexed with Lipofectamine® 2000 (Invitrogen™) according to the manufacturer's instructions, and 4) dPBS alone without m1Ψ-BMP-2 mRNA as a negative control.

The 30 µg of m1Ψ-BMP-2 mRNA was prepared in dPBS, sucrose citrate buffer or Lipofectamine® 2000. All rats received an intragingival injection of the solution of m1Ψ-BMP-2 mRNA with different carriers at the tissue on the palatal side, using needle gauge 31 under the loupe magnification. The injection was performed at six sites (Fig. 1A) with the volume of 6 µl solution, which contains 5 µg m1Ψ-BMP-2 mRNA, per site. A total volume of 36 µl solution was given to each rat.

Preparation of gingival tissue homogenates and measurement of protein expression *in vivo*

For analysis, the rats were sacrificed at 24-hr post-injection. Palatal gingival tissues were collected by #15C blade (Fig. 1B). The gingival tissues were weighed and homogenized with a Dounce glass homogenizer in phosphate-buffered saline (PBS) supplemented with 0.05% Tween-20, phenylmethyl sulfonyl fluoride (1 mmol/L; Sigma, St. Louis, MO, USA), and protease inhibitor cocktail (Sigma, St. Louis, MO, USA).[29] The homogenates were centrifuged, and the supernatants were collected for further protein analysis.

The total protein in tissue homogenates were measured using a BCA protein assay kit (Pierce™ BCA Protein Assay; Thermo scientific, Co., Ltd., Rockford, IL USA). The production of human BMP-2 protein and pro-inflammatory cytokines including rat interleukin-1β (IL-1β), rat tumor necrosis factor-α (TNF-α), and rat interleukin-6 (IL-6) were determined by commercially available enzyme-linked immunosorbent assay (ELISA) kits (Quantikine® ELISA; R&D Systems, Inc., Minneapolis, MN, USA). The ELISA kits were performed according to the manufacturer's protocols. The results were presented as the amount of interested growth factor or proinflammatory cytokines per total protein.

Statistical analysis

The statistical software SPSS version 17.0 (SPSS Inc., Chicago, IL, USA) was used for data analysis. Normal distribution of data was evaluated by the Shapiro-Wilk normality test. The non-parametric Kruskal-Wallis test with Dunn's correction (followed by pairwise comparisons) was performed between groups and multiple groups comparisons, respectively. For all statistical analysis, a p-value less than 0.05 was considered statistically significant.

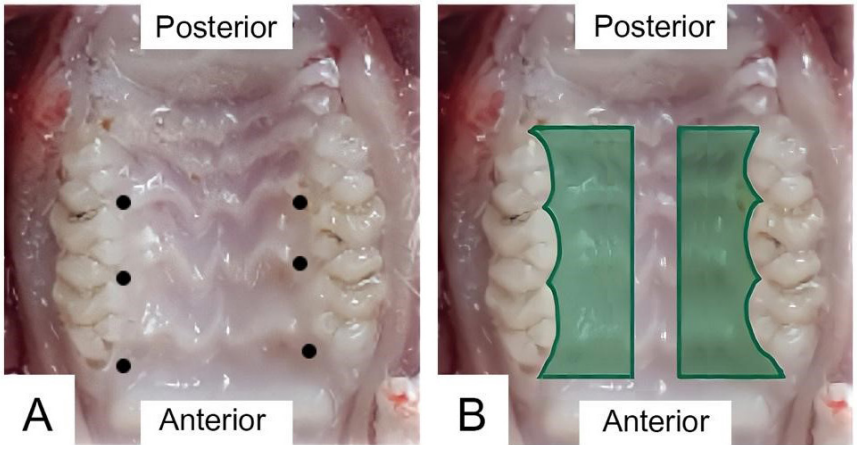

Figure 1 *Diagram of rat maxillary teeth: Gingival injection was performed 6 sites per rat. (A) Black dot indicated site for gingival injection. (B) Green line outlined the part of palatal gingiva that was collected*

Results

Mean BMP-2 protein levels in the sites injected with m1Ψ-BMP-2 mRNA regardless of the delivery system were significantly elevated after administration, compared with dPBS alone ($p<0.05$). The baseline level of BMP-2 protein in rat gingival tissues was very low and unable to be detected. The highest BMP-2 protein expression in gingival tissue was observed in a rat injected with m1Ψ-BMP-2 mRNA in Lipofectamine® 2000 (499.46 ± 108.61 pg/mg), followed by dPBS (308.62 ± 27.81 pg/mg) and sucrose citrate buffer (139.22 ± 125.85 pg/mg). However, only Lipofectamine® 2000 and sucrose citrate buffer showed a statistically significant difference among the three delivery systems ($p<0.05$) (Fig. 2A).

Furthermore, the effects of intragingival injection of m1Ψ-BMP-2 mRNA on the gingival levels of IL-1β, TNF-α and IL-6 was also evaluated. There was no statistically significant difference in gingival levels of IL-1β, TNF-α and IL-6 among the groups. In all the groups, the amount of IL-1β was less than 100 pg/mg of total protein (Fig. 2B). Neither TNF-α nor IL-6 was detected. Data was not shown.

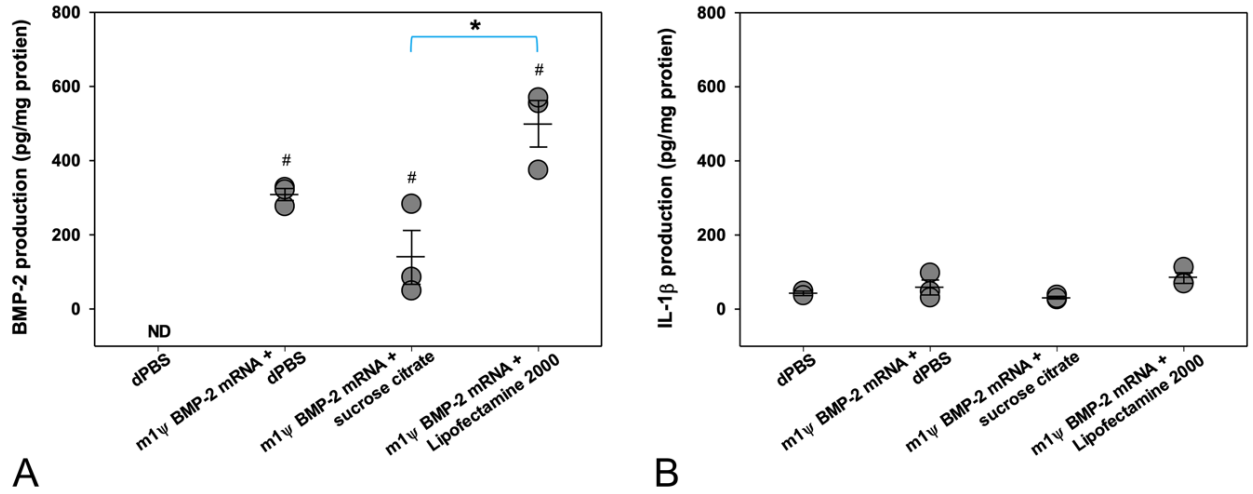

Figure 2 *In vivo production of (A) BMP-2 protein and (B) IL-1Ψ in rat gingiva at 24 hours after intragingival injection with dPBS alone and m1β-BMP-2 mRNA in different delivery systems. Data shown are mean ± SE (n=3, each dot represents the data of each rat, # significantly different compared to dPBS alone, * significant difference) (ND = not detected)*

Discussion

This is the first study demonstrated the *in vivo* expression of translated human BMP-2 upon intragingival injection with m1Ψ-BMP-2 mRNA. Superior protein level was observed when Lipofectamine® 2000 was used as a delivery system compared to sucrose citrate buffer and dPBS. The higher level of BMP-2 protein expression is probably due to lipid encapsulation that could promote mRNA stability, cellular uptake and prevent mRNA degradation by serum endonuclease.[30] In addition, low immunogenicity of m1Ψ-BMP-2 mRNA complexed with Lipofectamine® 2000 was demonstrated, as the amount of IL-1β was low and no measurable increase of TNF-α and IL-6 was detected. The larger number of protein samples could possibly increase the sensitivity of ELISA. However, the gingival tissues harvested from the rat were limited. Further investigations, such as a PCR assay, could be performed in order to determine the precise amount of pro-inflammatory cytokines production. These findings are in line with our previous *in vitro* study in human periodontal ligament cells (hPDL). When hPDL cells were transfected with m1Ψ-BMP-2 mRNA in Lipofectamine® 2000, the extracellular concentration of BMP-2 in supernatants collected from the transfected cells was higher compared to cells transfected with Lipofectamine® 2000 alone without an effect on cell viability.[31] Although the lipid-based delivery systems offer an efficient vehicle for mRNA delivery, toxicity regarding their use has been reported. Intracardiac delivery of mRNA with Lipofectamine® possibly increases apoptosis in cardiac cells around the injected site *in vivo* as compared to naked mRNA and mRNA in sucrose citrate buffer.[16] Additional experiments might be needed to verify the toxicity of Lipofectamine® 2000 when delivered intragingivally.

Few studies suggested that mRNA could be effectively delivered without the need for lipid carriers.[16,18] Sultana and colleague reported that intracardiac injection of modified mRNA encoding VEGF-A in sucrose citrate buffer in mice resulted in the highest protein translation when compared to other methods including lipid carrier.[16] Nevertheless, our results were in contrast to previous studies. We found that the level of human BMP-2 protein from m1Ψ-BMP-2 mRNA prepared in sucrose citrate buffer was not statistically different from the control. It is possible that the delivery of mRNA into different target tissues resulted in different patterns of translated protein expression. Therefore, more studies are required in order to identify the most efficient mRNA delivery systems that are both safe and lead to high protein production in gingival tissues.

mRNA technology could undoubtedly be a novel treatment platform for periodontal regeneration. In comparison to recombinant proteins, mRNAs is more stable, has a lower immunogenicity, a longer half-life, and lower production cost.[32] Furthermore, recombinant proteins could stimulate host immune responses, leading to rapid degradation and, thus, will need repetitive administration. Hence, the future direction in development of this mRNA therapeutic platform for periodontal treatment should focus on its pharmacokinetic. The kinetic properties of protein in terms of the duration of action and extent of production can be further investigated in order to optimize proper delivery systems to create a sustainable protein expression and to avoid possible complications. Moreover, mRNA technology should be tested in larger sample sizes to obtain essential information regarding the efficacy and safety of mRNA therapeutic platforms before being used in clinical trials.

Conclusion

This *in vivo* study has demonstrated the possibility of using intragingival injection of m1Ψ-BMP-2 mRNA to induce human BMP-2 proteins production in rat gingival tissue without stimulation local tissue inflammation. In comparison with sucrose citrate buffer and dPBS, m1Ψ-BMP-2 mRNA complexed with Lipofectamine® 2000 resulted in the highest amount of translated human BMP-2 protein expression. These preliminary data suggest the potential application of the IVT nucleoside-modified mRNA as a novel therapeutics platform for periodontal tissue regeneration. However, further studies with larger sample size are required.

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References

1. Kornman KS. Mapping the pathogenesis of periodontitis: a new look. ***J Periodontol*** 2008;79(8S):1560-8.

2. Sculean A, Nikolidakis D, Nikou G, Ivanovic A, Chapple IL, Stavropoulos A. Biomaterials for promoting periodontal regeneration in human intrabony defects: a systematic review. ***Periodontol 2000*** 2015;68(1):182-216.

3. Jepsen S, Schwarz F, Cordaro L, Derks J, Hämmerle CH, Heitz-Mayfield LJ, *et al.* Regeneration of alveolar ridge defects. Consensus report of group 4 of the 15th European Workshop on Periodontology on Bone Regeneration. ***J Clin Periodontol*** 2019;46:277-86.

4. Suárez-López del Amo F, Monje A, Padial-Molina M, Tang Z, Wang H-L. Biologic agents for periodontal regeneration and implant site development. ***Biomed Res Int*** 2015;2015:957518.

5. Oortgiesen DA, Walboomers XF, Bronckers AL, Meijer GJ, Jansen JA. Periodontal regeneration using an injectable bone cement combined with BMP-2 or FGF-2. ***J Tissue Eng Regen Med*** 2014;8(3):202-9.

6. Sorensen RG, Wikesjö UM, Kinoshita A, Wozney JM. Periodontal repair in dogs: evaluation of a bioresorbable calcium phosphate cement (CeredexTM) as a carrier for rhBMP-2. ***J Clin Periodontol*** 2004;31(9):796-804.

7. Blumenthal NM, Koh-Kunst G, Alves ME, Miranda D, Sorensen RG, Wozney JM, *et al.* Effect of surgical implantation of recombinant human bone morphogenetic protein-2 in a bioabsorbable collagen sponge or calcium phosphate putty carrier in intrabony periodontal defects in the baboon. ***J Periodontol*** 2002;73(12):1494-506.

8. Kao RT, Nares S, Reynolds MA. Periodontal regeneration–intrabony defects: a systematic review from the AAP regeneration workshop. ***J Periodontol*** 2015;86:S77-S104.

9. Dunbar CE, High KA, Joung JK, Kohn DB, Ozawa K, Sadelain M. Gene therapy comes of age. ***Science*** 2018;359(6372):eaan4672.

10. Hajj KA, Whitehead KA. Tools for translation: non-viral materials for therapeutic mRNA delivery. ***Nat Rev Mater*** 2017;2(10):1-17.

11. Karikó K, Buckstein M, Ni H, Weissman D. Suppression of RNA recognition by Toll-like receptors: the impact of nucleoside modification and the evolutionary origin of RNA. ***Immunity*** 2005; 23(2):165-75.

12. Andries O, McCafferty S, De Smedt SC, Weiss R, Sanders NN, Kitada T. N1-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.

13. Mintzer MA, Simanek EE. Nonviral vectors for gene delivery. ***Chem Rev*** 2009;109(2):259-302.

14. Mandal PK, Rossi DJ. Reprogramming human fibroblasts to pluripotency using modified mRNA. ***Nat Protoc*** 2013;8(3):568.

15. Umrath F, Steinle H, Weber M, Wendel H-P, Reinert S, Alexander D, *et al.* Generation of iPSCs from jaw periosteal cells using self-replicating RNA. ***Int J Mol Sci*** 2019;20(7):1648.

16. Sultana N, Magadum A, Hadas Y, Kondrat J, Singh N, Youssef E, *et al.* Optimizing cardiac delivery of modified mRNA. ***Mol Ther*** 2017; 25(6):1306-15.

17. Magadum A, Singh N, Kurian AA, Sharkar MTK, Chepurko E, Zangi L. Ablation of a single N-glycosylation site in human FSTL 1 induces cardiomyocyte proliferation and cardiac regeneration. ***Mol Ther Nucleic Acids*** 2018;13:133-43.

18. Carlsson L, Clarke JC, Yen C, Gregoire F, Albery T, Billger M, *et al.* Biocompatible, purified VEGF-A mRNA improves cardiac function after intracardiac injection 1 week post-myocardial infarction in swine. ***Mol Ther Methods Clin Dev*** 2018;9:330-46.

19. 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.

20. Khorsand B, Elangovan S, Hong L, Dewerth A, Kormann MS, Salem AK. A comparative study of the bone regenerative effect of chemically modified RNA encoding BMP-2 or BMP-9. ***AAPS J*** 2017;19(2):438-46.

21. Yu Z, Witman N, Wang W, Li D, Yan B, Deng M, *et al.* Cell-mediated delivery of VEGF modified mRNA enhances blood vessel regeneration and ameliorates murine critical limb ischemia. ***J Control Release*** 2019;310:103-14.

22. Almquist J, Rikard SM, Wågberg M, Bruce AC, Gennemark P, Fritsche-Danielson R, *et al.* Model-Based Analysis Reveals a Sustained and Dose-Dependent Acceleration of Wound Healing by VEGF-A mRNA (AZD8601). ***CPT Pharmacometrics Syst Pharmacol*** 2020; 9(7):384-94.

23. Rizvi F, Everton E, Smith AR, Liu H, Osota E, Beattie M, *et al.* Murine liver repair via transient activation of regenerative pathways

in hepatocytes using lipid nanoparticle-complexed nucleoside-modified mRNA. ***Nat Commun*** 2021;12(1):1-10.
24. Szőke D, Kovács G, Kemecsei É, Bálint L, Szoták-Ajtay K, Aradi P, *et al.* Nucleoside-modified VEGFC mRNA induces organ-specific lymphatic growth and reverses experimental lymphedema. ***Nat Commun*** 2021;12(1):1-18.
25. Gan L-M, Lagerström-Fermér M, Carlsson LG, Arfvidsson C, Egnell A-C, Rudvik A, *et al.* Intradermal delivery of modified mRNA encoding VEGF-A in patients with type 2 diabetes. ***Nat Commun*** 2019;10(1):1-9.
26. 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.
27. Pardi N, Secreto AJ, Shan X, Debonera F, Glover J, Yi Y, *et al.* Administration of nucleoside-modified mRNA encoding broadly neutralizing antibody protects humanized mice from HIV-1 challenge. ***Nat Commun*** 2017;8(1):1-8.
28. 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.
29. Kawai T, Matsuyama T, Hosokawa Y, Makihira S, Seki M, Karimbux NY, *et al.* B and T lymphocytes are the primary sources of RANKL in the bone resorptive lesion of periodontal disease. ***Am J Pathol*** 2006;169(3):987-98.
30. Kowalski PS, Rudra A, Miao L, Anderson DG. Delivering the messenger: advances in technologies for therapeutic mRNA delivery. ***Mol Ther*** 2019;27(4):710.
31. Kulthanaamondhita P, Champaiboon C, Sa-Ard-Iam N, Rerkyen P, Chanamuangkon T, Mahanonda R. Utilization of modified mRNA encoding bone morphogenetic protein-2 for periodontal regeneration: an *in vitro* study. Proceedings of RSU International Research Conference (2020) Pathum Thani, Thailand. 2020.
32. Magadum A, Kaur K, Zangi L. mRNA-based protein replacement therapy for the heart. ***Mol Ther*** 2019;27(4):785-93.