ABSTRACT

Objectives: To investigate the effect of using osteogenic induced gingival fibroblasts (OIGFs) and low intensity pulsed ultrasound (LIPUS) on root resorption lacunae volume and cementum thickness in beagle dogs that received orthodontic tooth movement.

Materials and Methods: Seven beagle dogs were used, from which gingival cells (GCs) were obtained and were induced osteogenically to produce OIGFs. Each third and fourth premolar was randomly assigned to one of the five groups, namely, LIPUS, OIGFs, bone morphogenetic protein-2 (BMP-2), OIGFs + LIPUS, and control. All groups received 4 weeks of bodily tooth movement; then LIPUS-treated groups received LIPUS for 20 min/day for 4 weeks, and OIGFs groups received an injection of OIGFs near the root apex. Microcomputed tomography analysis was used to calculate root resorption lacunae volume and histomorphometric analysis was performed to measure the cementum thickness of each root at 3 root levels on compression and tension sides.

Results: There was no significant difference in resorption volume between the treatment groups. OIGFs + LIPUS increased cementum thickness ($P > 0.05$) in third premolars near the apex, and LIPUS increased cementum thickness ($P > 0.05$) in fourth premolars near the apex. Furthermore, BMP2 increased cementum thickness at the coronal third at the compression side.

Conclusion: OIGFs, LIPUS, and BMP-2 can be potential treatments for orthodontically induced root resorption, however, improvements in experimental design and treatment parameters are required to further investigate these repair modalities.

Key words: Dental cementum, gingival fibroblasts, root resorption, ultrasound

INTRODUCTION

A favorable tooth crown-to-root ratio is important to support a tooth. Severe root resorption and/or resorbed alveolar bone adversely affect this ratio by shortening the root that is invested in the alveolar bone.[1] A report on orthodontically induced tooth root resorption (OIRR) revealed that 40% adults had at least one tooth with 2.5 mm of resorption.[2] OIRR can take place within 35 days of orthodontic treatment even with forces as light as 50 g.[3]

OIRR is a pathological process resulting in cementum and dentin loss.[1] The original root contours cannot be reconstructed
after this type of root resorption has occurred. Low intensity pulsed ultrasound (LIPUS) can prevent the progression of OIRR if discovered early or during orthodontic treatment, but no treatment is available to repair severe OIRR. A new technique is needed to be developed to repair the lost root parts after OIRR.

An interest in using stem cells for regenerating dental tissues has risen recently. Human PDL stem cells are capable of repairing PDL defects in mice and rats, and in dogs. PDL fibroblast-like cells can prevent root resorption and induce cementum formation in dogs. PDL cells can be differentiated into osteogenic, adipogenic, and neural phenotypes. Current techniques that use stem cells in root repair have achieved preliminary successes. However, they suffer from drawbacks such as donor-site morbidity. Better sources of stem/pluripotent cells are needed for PDL tissue repair and OIRR treatments.

Gingival cells/fibroblasts (GCs/GFs) show promise in dental repair due to their accessibility. GCs can enhance vascularization and improve attached gingiva, inhibit osteoclast activity, have neural differentiation potential, and be induced into osteogenic cells.

Other techniques concerning repair of dental tissues involve the use of a variety of growth factors such as bone morphogenetic protein-2 (BMP-2). Previous studies showed an increase in cementum formation in root defects after application of BMP-2, and that BMP-2 plays a role in increasing alkaline phosphatase activity, leading to an increased mineralization activity of cells. LIPUS is acoustic pressure waves transmitted through living tissues. LIPUS can enhance PDL cell differentiation into cementoblast-like cells, increase cellular proliferation and induce osteogenic differentiation in GFs, and enhance repair of OIRR during orthodontic treatment in dogs.

The aim of this study was to analyze the possible effect of osteogenic-induced gingival fibroblasts (OIGFs) and LIPUS on cementum in beagle dogs undergoing orthodontic treatment. It was hypothesized that OIGFs and LIPUS would help repair roots damaged by OIRR, and that the repair effects of OIGFs would be complementary to those of LIPUS when OIGFs and LIPUS are applied in combination. It was also hypothesized that BMP-2 would help repair root resorption and that this effect would be comparable to those of LIPUS and OIGFs.

**MATERIALS AND METHODS**

**Animals and Gingival Cells**

Seven beagle dogs (aged 19 months ± 8 days) were used in the study. The rights of the animals were protected, and the experimental procedure was approved by the Animal Care Committee at the University of Alberta. GCs were isolated and induced for osteogenic differentiation as described before. In short, interdental papilla from each dog was excised, cut, and dispersed on slides, placed in culture plates, and incubated. The cells that became confluent (2–3 weeks) were removed from the plates and were transferred into flasks. The cultured GFs (cells) were transferred to 48-well plates (2.5 × 10⁴ cells/well), treated with osteogenic medium (basic medium, 10 mM β-glycerophosphate, 50 mg/L ascorbic acid, and 0.1 μM dexamethasone), and received LIPUS treatment for 20 min/day for 4 weeks according to the described protocol to produce OIGFs. LIPUS was applied with 30 mW/cm² intensity pulsed at 1.5 MHz and repeated at a frequency of 1 kHz.

**Orthodontic Tooth Movement and Treatment Groups**

Bodily orthodontic tooth movement was performed to move 3rd premolars mesially and 4th premolars slightly distally for 4 weeks using 100 cN force (RMO, Denver, CO, USA), as described before. Premolars were randomly assigned to one of five treatment groups [Table 1]. Treatments were performed after 4 weeks of tooth movement. OIGFs groups were injected transosseously (Dentsply™ X-Tip Intraosseous Anesthetic Delivery System, Pennsylvania) with 0.5 mL of OIGFs (in DMEM) from each dog into the same dog through the buccal plate of bone near the apex using a 30-gauge needle. The concentration of cells was 2 × 10⁵ cells/mL of DMEM, and the viability of OIGFs after passing through the needle was confirmed prior to the actual injection. LIPUS treatments were applied for 20 min/day for a total of 4 weeks. BMP-2 (Julius-Maximilians-Universität Würzburg) was conjugated in poly-D, L-lactic acid-polyethylene glycol (PLA-PEG) polymer (25 mg of PLA-PEG polymer mixed with 10 μL of BMP-2 in buffered solution per injection) was injected through the buccal plate of bone near the apex of each corresponding tooth. This procedure has been described by Saito et al. BMP-2 was used as a positive control. After 4 weeks, the animals were euthanized with ketamine HCl overdose injected intravenously according to the approved standard operating procedures.

**Histology and Histomorphometric Analysis**

Tissue blocks were dissected and stored in 4% paraformaldehyde. After the samples were air dried for 30 minutes, microcomputed tomography scanning was performed using SkyScan® 1076 MicroCT scanner and associated software (Version 2.6.0). Reconstructed images were created, and resorption lacunae volumes were measured using CTAn software (SkyScan®)

**Table 1: Number of premolars included in each treatment group**

| Treatment Groups | Sample Size (Number of premolars) |
|------------------|-----------------------------------|
| Control          | 6                                 |
| LIPUS            | 5                                 |
| OIGF             | 6                                 |
| BMP2             | 5                                 |
| OIGF+LIPUS       | 6                                 |
according to the method described before [Figure 1]. The total resorption lacunae volume was calculated for each tooth root in each treatment group and then compared. Tissue blocks were prepared for histomorphometric analysis by demineralizing in 10% formic acid and decalcifying in ethylenediaminetetraacetic acid (EDTA), then cutting serial sections (7 μm) in the buccolingual plane through mesiodistal extension and staining with hematoxylin and eosin. Slides were analyzed by light microscopy (Leica Qwin 500 image analyzer computer system [England]) at 40× magnification and photos were then produced. MetaMorph Software (Molecular Devices LLC, California) was used to measure cementum thickness on compression and tension sides at 3 root levels, namely, coronal (level 1), middle (level 2), and apical (level 3) [Figure 2]. Sample photomicrographs and micro-CT images from a tooth root from each treatment group and the control group were produced [Figure 3]. The assessor was blinded during all data collection.

**Statistical Analysis**

Normal distribution of data was assessed using Kolmogorov–Smirnov tests of normality and box-plots. Cementum thickness data that was not normally distributed was transformed using the natural logarithm (x + 1) [i.e., Ln (thickness + 1)] in order to obtain normal distribution. Root resorption volume data that was not normally distributed was also transformed using the natural logarithm (x + 1) [i.e., Ln (volume + 1)] because some tooth roots contained no resorption. After data transformation, significant differences (P < 0.05) were calculated using analysis of variance (ANOVA) with least squares difference (LSD) post-hoc tests for normally distributed data and using Kruskal–Wallis test with Tukey post-hoc tests for data that remained non-normally distributed after transformation. Levene’s test of variance was performed to determine differences in cementum thickness within populations of third and fourth premolars in the control group in order to provide a substitute for baseline measurements when determining the effect of treatment on tooth roots. To consider the possibility of cross-contamination of LIPUS treatment on tooth roots treated with OIGFs and roots treated with BMP-2 because of their location immediately beside LIPUS-treated roots, cementum thicknesses and root resorption lacunae volumes of possibly cross-contaminated roots were compared with tooth roots that were not located immediate to LIPUS-treated roots. Data that was normally distributed was statistically analyzed using independent t-tests and data that was not normally distributed was analyzed using Mann–Whitney U tests. Intraclass correlation coefficients (ICCs) were calculated for cementum thickness in each treatment group and for root resorption volume by randomly re-measuring five samples and five roots, respectively, at least 8 weeks after the original measurements.

**RESULTS**

Figure 4 shows the comparison of the root resorption volumes of each tooth root in each treatment group. Figure 5 shows the comparison of cementum thickness between groups. Levene’s test of variance shows that there was no significant difference within populations of third and fourth premolars in the control group [Table 2]. Comparison of root resorption volumes and cementum thicknesses between teeth, which were expected to have some sort of cross contamination between them, are presented in Figures 6-8. ICCs were calculated [Table 3] and were at least 0.80 (strong agreement).

**DISCUSSION**

To the best of our knowledge, this is the first study to evaluate the effect of a transosseous injection of OIGF and LIPUS on
orthodontically induced root resorption in beagle dogs. The present study tested the hypothesis that an intraosseous injection of OIGFs and application of LIPUS for 4 weeks can enhance OIRR repair by decreasing root resorption volume and by increasing cementum thickness, which may be interpreted as regaining resorbed root volume. In orthodontics, a complication of tooth movement is root resorption, also known as apical root resorption, which is an injury resulting from pressure applied to tooth roots during orthodontic treatment. This continuous orthodontic pressure stimulates the activity of resorbing cells, known as osteoclasts, and increases the possibility of shortening the tooth root.\(^{[30]}\) Although it is important to analyze the whole tooth root when considering root resorption, focus should be placed on damage to the apical third of the root, since resorbing of dental cementum in this location leads to this root shortening.\(^{[30]}\) However, the present study employed bodily tooth movement with the intention of homogeneously distributing orthodontic force along the tooth root. Although this type of tooth movement is better at uniformly applying pressure in a more diffuse and less concentrated manner, there will always be some degree of tipping movement, which tends to concentrate forces on apical and cervical regions and is more associated with apical root resorption.\(^{[31]}\) Therefore, it is expected in the present study that more root resorption would have resulted near the apex, but not to the extent as it would have been if tipping movement was used.

This study first measured root resorption lacunae on tooth roots in every treatment group. The sums of resorption lacunae on each root were then used in comparing root resorption volumes in each treatment group in order to determine the possible effect of treatment on reducing this volume. Figure 4 shows that the OIGF group had the greatest root resorption volume in fourth premolars but the least root resorption volume in third premolars. The OIGF + LIPUS group had the least lacunae

---

**Table 3:** Intraclass correlation coefficients for resorption lacunae volume and cementum thickness for each group

|          | Cementum Thickness | Resorption Volume |
|----------|--------------------|-------------------|
| Control  | 0.993              |                   |
| LIPUS    | 0.999              |                   |
| OIGF     | 0.984              |                   |
| BMP2     | 0.911              |                   |
| OIGF+LIPUS | 0.997            | 0.8               |

LIPUS – Low intensity pulsed ultrasound; OIGF – Osteogenic induced gingival fibroblasts; BMP2 – Bone morphogenetic protein-2
Figure 5: Cementum thickness (μm) for each group in third and fourth premolars on compression side (a) and tension side (b) of the root at three root levels (1 = coronal, 2 = middle, 3 = apical). * = \( P < 0.05 \), ** = \( P < 0.01 \), *** = \( P < 0.005 \)

Figure 6: Root resorption volume (mm³) of third and fourth premolars in OIGFs and BMP2 groups that contain possible cross-contamination from LIPUS treatment and groups that do not have possible cross-contamination from LIPUS

Because this study did not include pretreatment measurements of cementum thickness, the Levene’s test of equal variance was performed to determine differences within each population of third and fourth premolars in the control group. Table 2 shows that there was no difference within populations of third and fourth premolars in the control group (\( P > 0.05 \)). This conclusion can be used in place of baseline measurements to analyze the effect of treatment on tooth roots.

Figure 5 shows that, although on third premolars cementum thickness was greatest in the control group (\( P < 0.05 \)) at the middle level on both compression and tension sides, the OIGF + LIPUS group had the greatest cementum thickness compared to the other groups at the apical level on both sides of the root. However, this group was not significantly different from the other treatment groups.

During this experiment, the intention was to move third premolars mesially and fourth premolars distally. However, fourth premolars were prevented from moving distally as much as expected due to the first molars being distal and more proximal to the fourth premolars than the second premolars were to the third premolars. Because of this, fourth premolars would have decreased compression and tension sides compared to the third premolars, and it is reasonable to expect different results from treatments on fourth premolars. Figure 4 shows that OIGF + LIPUS groups on the compression side of fourth premolar roots resulted in the thinnest cementum at each root level; however, this difference was not statistically significant at the middle level and near the apex. LIPUS treatment appeared to have greater effect on increasing cementum thickness on the tension side of fourth premolar...
Crossman, et al.: Effect of gingival fibroblasts and LIPUS on root resorption

It is interesting to note that BMP-2 increased cementum thickness at each root level on both sides of the fourth premolar root in comparison to the OIGF + LIPUS group. Our results are in agreement with a previous study that showed that the application of BMP-2 to tooth root defects resulted in cementum-like tissue formation compared to control root defects. Upon analysis of the results, it was speculated that some tooth roots treated with BMP-2 may have been cross-contaminated by treatment with LIPUS because some LIPUS-treated roots were located immediately beside BMP-2-treated roots. To investigate this speculation, root resorption volume and cementum thickness (at each root level on compression and tension sides) were compared among BMP-2-treated roots located immediate to LIPUS-treated roots and BMP-2-treated roots not beside roots receiving LIPUS treatment. Figure 6 shows that there was no significant difference in resorption volume between third premolars with the possibility of cross-contamination and third premolars without cross-contamination, and between fourth premolars with the possibility of cross-contamination and fourth premolars without cross-contamination. Figure 7 shows that BMP-2-treated tooth roots with possible cross-contamination of LIPUS had thinner cementum at almost all root levels compared to those without cross-contamination.
roots without cross-contamination, however, no statistically
significant differences were calculated. Sant’Anna et al.[32]
investigated in vitro the effect of LIPUS applied in combination
with BMP-2 treatment on the expression of genes associated
with osteogenesis in rat stromal cells. They found that there
was no additive or synergistic effect of the combination of these
two treatments. Because in the present study, the exposure
of LIPUS treatment on BMP-2-treated tooth roots was only
through possible cross-contamination, it may be possible
that this LIPUS exposure was at a lower intensity compared
to direct application as in LIPUS- and OIGF + LIPUS-treated
roots due to slight dissipation of LIPUS through tissue. Direct
application of LIPUS to BMP-2-treated roots at a higher intensity
or using more optimal levels may lead to a synergistic effect,
but it may be possible that in the present study this continual
decreased exposure may have had a negative effect of
BMP-2 on cementum thickness. It is suspected that, if possible
cross-contamination of LIPUS on BMP-2-treated roots was absent,
the overall effect of this treatment on cementum
thickness may have been significantly greater than what is
demonstrated in the present study.

Similarly, it was also suspected that LIPUS cross-contaminated
OIGF-treated tooth roots. The possibility of cross-contamination
of LIPUS on these tooth roots appears to have had a positive
effect on OIGF treatment on resorption volume, but these
differences are statistically significant ($P < 0.01$) [Figure 8]. This
figure demonstrates that possibly-contaminated OIGF-treated
roots had greater cementum thickness at the apical third of the
root on the both sides of third and fourth premolars with a
significant difference calculated on the compression side of
third premolars. El-Bialy et al.[33] investigated the difference
between human and dogs’ gingival mesenchymal cells in vitro.
This study demonstrated that canine gingival mesenchymal
cells (CGMCs) responded differently than human gingival
mesenchymal cells (HGMCs) when both types of cells were
grown in osteogenic medium and received LIPUS treatment
for 1 day. Extensive research has been performed to show the
anabolic effect of LIPUS on different types of cells, including
gingival cells,[34] however, research performed on CGMCs is
limited. El-Bialy et al.[33] showed that it may be possible that
CGMCs require alternative parameters of LIPUS treatment,
such as intensity and length of exposure, compared to
parameters used currently in the treatment of HGMCs. This may
explain the overall decreased effect of OIGF and OIGF + LIPUS
treatment.

Future studies should not allocate different treatments
to neighboring teeth in order to avoid the possibility of
cross-contamination of treatments, especially those that can
dissipate through tissues, such as ultrasound. This study
also did not biologically track the injected OIGFs. Biologically
labelling these cells would allow tracking of these cells to be
determined and to know whether or not if these cells became
incorporated into the target tissue and used in tissue repair.
Finally, the present study used identical ultrasound application
parameters that are used in studies involving human gingival
cells. Future studies may also be directed to understanding the
possible mechanisms, of which OIGF, LIPUS and BMP2 might
be involved in repairing OITRR and possible interrelationship
between these mechanisms for possible optimum combination
of these treatment modalities in treating/prevention of OITRR.

CONCLUSION

This study showed that OIGF, LIPUS, and BMP-2 may have a
possible effect on the repair of OITRR, however, more optimal
parameters of ultrasound use and improved experimental
design are required in order to further investigate this effect.

Acknowledgements

This research was supported by the Qatar National Research
Fund, NPRP grant number NPRP No. 09-557-3 – 144.

Financial Support and Sponsorship

Nil.

Conflicts of Interest

There are no conflicts of interest.

REFERENCES

1. Cwyk F, Scat-Pierre F, Tronstad L. Endodontic implications of
orthodontic tooth movement. J Dent Res 1984;63:Abstract1039.
2. Mirabella AD, Artun J. Prevalence and severity of apical root resorption
of maxillary anterior teeth in adult orthodontic patients. Eur J Orthod
1995;17:93-9.
3. Harry MR, Sims MR. Root resorption in bicuspid intrusion. A scanning
electron microscope study. Angle Orthod 1982;2:235-58.
4. Bosshardt DD, Schroeder HE. How repair cementum becomes
attached to resorbed roots of human permanent teeth. Acta Anat
1994;150:253-366.
5. Remington D, Joondeph D, Artun J, Riedel R, Chapko M. Long-term
evaluation of root resorption occurring during orthodontic treatment.
Am J Orthod Dentofacial Orthop 1989;96:43-6.
6. Brezniau N, Wasserstein A. Orthodontically induced inflammatory
root resorption. Part I: The basic science aspects. Angle Orthod
2002;72:175-9.
7. El-Bialy T, El-Shamy I, Graber TM. Repair of orthodontically induced
root resorption by ultrasound in humans. Am J Orthod Dentofacial
Orthop 2004;126:186-93.
8. Lin NH, Gronthos S, Bartold PM. Stem cells and periodontal
regeneration. Aust Dent 2008;53:10821.
9. Silvério KG, Benatti BB, Casati MZ, Sallum EA, Nociti FH Jr. Stem cells:
Potential therapeutics for periodontal regeneration. Stem Cell Rev
2008;4:13-9.
10. Ferreira CF, Magini RS, Sharpe PT. Biological tooth replacement and
repair. J Oral Rehabil 2007;34:933-9.
11. Soo BM, Miura M, Gronthos S, Bartold PM, Batouli S, Brahim J,
et al. Investigation of multipotent postnatal stem cells from human
periodontal ligament. Lancet 2004;364:149-55.
12. Kim SH, Kim KH, Seo BM, Koo KT, Kim TI, Seol YJ, et al. Alveolar bone
regeneration by transplantation of periodontal ligament stem cells
and bone marrow stem cells in a canine peri-implant defect model:
A pilot study. J Periodontol 2009;80:1815-23.
13. Doğan A, Ozdemir A, Kubar A, Oygür T. Assessment of periodontal
healing by seeding of fibroblast-like cells derived from regenerated
periodontal ligament in artificial furcation defects in a dog: A pilot
study. Tissue Eng 2002;8:273-82.
14. Zhou Y, Hutmacher DW, Sae-Lim V, Zhou Z, Woodruff M, Lim TM. Osteogenic and adipogenic induction potential of human periodontal cells. J Periodontol 2008;79:525-34.

15. Widera D, Grimm WD, Moebius JM, Mikenberg I, Piechaczek C, Gassmann G, et al. Highly efficient neural differentiation of human somatic stem cells, isolated by minimally invasive periodontal surgery. Stem Cells Dev 2007;16:447-60.

16. Mohammadi M, Shokrgozar MA, Mofid R. Culture of human gingival fibroblasts on a biodegradable scaffold and evaluation of its effect on attached gingiva: A randomized, controlled pilot study. J Periodontal 2007;78:1897-903.

17. De Vries TJ, Schoenmaker T, Wattanaroonwong N, van den Hoonaard M, Nieuwenhuijse A, Beertsen W, et al. Gingival fibroblasts are better at inhibiting osteoclast formation than periodontal ligament fibroblasts.

18. El-Bialy T, Alhadlaq A, Wong B, Kucharski C. Ultrasound effect on neural differentiation of gingival stem/progenitor cells. Ann Biomed Eng 2014;42:1406-12.

19. Mostafa N, Scott P, Dederich DN, Doschak M, El-Bialy T. Low intensity pulsed ultrasound stimulates osteogenic differentiation of human gingival fibroblasts. Can Acoustics 2008;36:34-5.

20. Miyaji H, Sugaya T, Ike K, Ishizuka R, Tokunaga K, Kawanami M. Root surface conditioning with bone morphogenetic protein-2 facilitates cementum-like tissue deposition in beagle dogs. J Periodontal Res 2010;45:658-63.

21. Neve A, Corrado A, Cantatore F. Osteoblast physiology in normal and pathological conditions. Cell Tissue Res 2011;343:289-302.

22. Inubushi T, Tanaka E, Rego EB, Kitagawa M, Kawazoe A, Ohta A, et al. Effects of ultrasound on the proliferation and differentiation of cementoblast lineage cells. J Periodontal 2008;79:1984-90.

23. Doan N, Reher P, Meghji S, Harris M. In vitro effects of therapeutic ultrasound on cell proliferation, protein synthesis, and cytokine production by human fibroblasts, osteoblasts, and monocytes. J Oral Maxillofac Surg 1999;57:409-19.

24. Al-Dagher S, Doschak M, Sloan A, Major P, Heo G, Scurtescu C, et al. Effect of low-intensity pulsed ultrasound on orthodontically induced root resorption in beagle dogs. Ultrasound Med Biol 2014;40:1187-96.

25. De Vasconcellos LM, Ricardo LH, Balducci I, de Vasconcellos LG, Carvalho YR. Histological analysis of effects of 24% EDTA gel for nonsurgical treatment of periodontal tissues. J Oral Sci 2006;48:207-14.

26. Maltha JC, van Leeuwen EJ, Dijkman GE, Kuijpers-Jagtman AM. Incidence and severity of root resorption in orthodontically moved premolars in dogs. Orthod Craniofac Res 2004;7:115-21.

27. Follin ME, Ericsson I, Thilander B. Occurrence and distribution of root resorption in orthodontically moved premolars in dogs. Angle Orthod 1986;56:164-75.

28. Van Leeuwen EJ, Maltha JC, Kuijpers-Jagtman AM. Tooth movement with light continuous and discontinuous forces in beagle dogs. Eur J Oral Sci 1999;107:468-74.

29. Saito N, Okada T, Horichi H, Ota H, Takahashi J, Murakami N, et al. Local bone formation by injection of recombinant human bone morphogenetic protein-2 contained in polymer carriers. Bone 2003;32:381-6.

30. Fuss Z, Tsesis I, Lin S. Root resorption – diagnosis, classification and treatment choices based on stimulation factors. Dent Traumatol 2003;19:175-82.

31. Consolaro A. Force distribution is more important than its intensity. Dental Press J Orthod 2014;19:5-7.

32. Sant’Anna E, Leven R, Virdi A, Sumner D. Effect of low intensity pulsed ultrasound and BMP-2 on rat bone marrow stromal cell gene expression. J Orthop Res 2005;23:646-52.

33. Inubushi T, Kucharski C, Farid M, Abdel Ghaffar K, Fawzi E, Saleem A. Human and dogs’ gingival stem cells are different. Int J Stem Cell Res 2015;1:1-5.

34. Shiraishi R, Masaki C, Toshinaga A, Okinaga T, Nishihara T, Yamanaka N, et al. The effects of low-intensity pulsed ultrasound exposure on gingival cells. J Periodontal 2011;82:1498-503.