TRIDHA
Journal of Clinical Cases & Reports
Volume 2022, Issue 2, 9 pages
https://doi.org/10.46619/joccr.2022.5-2.1110

CLINICAL RESEARCH

The Impacts of Orthodontic Force and Type of Bone Substitute on Nuclear Factor-kB Ligand and Osteoprotegerin Expression

Ferdinand Mabula Machibya[1*], Yiyuan Zhuang[2] and Jiang Chen[2]

[1]*Department of Orthodontics, Pedodontics and Community Dentistry, Muhimbili University of Health and Allied Sciences, Tanzania*
[2]*Implantology Department, School of Stomatology, Fujian Medical University, PR China*

Correspondence should be addressed to Ferdinand Mabula Machibya, Department of Orthodontics, Pedodontics and Community Dentistry, Muhimbili University of Health and Allied Sciences, Tanzania

Received: 11 November 2021; Accepted: 17 November 2021; Published: 23 November 2021

ABSTRACT

OBJECTIVE

To assess the impacts of orthodontic force and type of bone substitute on nuclear factor-kB ligand and osteoprotegerin expression.

METHODOLOGY

The expression of OPG and RANKL in the gingival crevicular fluid (GCF) collected before and at the end of OTM was examined by Enzyme linked immunosorbent assay to determine the impacts of regeneration materials and orthodontic force on concentration of the two biomarkers in GCF.

RESULTS

The OPG concentration registered at BL and end of OTM in experimental group was statistically significant different ($p < 0.05$) and the OPG concentration Before OTM was significantly higher in the control's than the Bio Oss group ($p < 0.05$).

The RANKL concentrations registered at BL and end of OTM within groups were statistically significant different in all groups ($p < 0.01$) and the Control group had consistently lower concentration than experimental groups at BL and end of OTM. There was no RANKL concentration difference between groups at BL, but the β-TCP group had statistically higher concentration than control and Bio Oss groups at the end of OTM ($p < 0.05$).

The Bio Oss registered significantly higher ratio than both control and β-TCP at BL: However, the ratio was significantly higher in β-TCP compared to Bio Oss group at the end of OTM ($p < 0.05$).

CONCLUSION

The orthodontic mechanical force as well as the type of BRMs significantly affected the RANKL and OPG concentration in GCF reflecting impact of biomechanical force and type of BRMs on biological activities at the site of bone regeneration.

KEYWORDS

Bone regeneration; Orthodontic tooth movement; Bone substitute

INTRODUCTION

The movement of a tooth occurs due to the translocation of the tooth from one position in the jaw to another. Extrinsic forces applied to the crown of the tooth during physiological, therapeutic, or pathological processes cause tooth movement [1]. Tumour Necrosis Factor alpha (TNF a), is an inflammatory cytokine produced by macrophages/monocytes during acute inflammation and is responsible for a diverse range of signalling events within cells including bone resorption by osteoclasts. The Receptor Activator of Nuclear factor Kappa-B Ligand (RANKL) is a member of the tumor necrosis factor (TNF) cytokine family which is a ligand for osteoprotegerin (OPG) and functions as a key factor for osteoclast differentiation and activation [2]. The orthodontic biomechanical force activates osteoblasts. In response, osteoblasts produce a number of molecules including bone morphogenetic proteins (BMPs), (RANKL), macrophage colony stimulating factor (M-CSF), osteoprotegerin (OPG), heat shock protein (HSP), transcription factors (osterix, Run X-2), fibroblast growth factor (FGF), platelet derived growth factor (PDGF), epidermal growth factor (EGF), insulin growth factor (IGF). BMP-2. transforming growth factor beta (TGF beta) and BMP-7 are involved in osteoblast differentiation. Each molecule has a specific role to play in the complex signalling network [2,3]. All cellular activities in the periodontium are regulated by multiple molecules and mechanisms. The basic functions of these molecules and pathways are to activate and regulate cell growth, proliferation, migration, differentiation, gene expression and cell functions and remodel ECM, PDL, and alveolar bone.

The RANK/RANKL/OPG signalling pathway is essential for oesteoclastogenesis. This signalling pathway is inhibited by the binding of OPG to RANKL. Osteoprotegerin (OPG) is a decoy receptor for the receptor activator of nuclear factor kappa B ligand (RANKL). By binding RANKL, OPG inhibits nuclear kappa B (NF-κB) [4]. Thus, Osteoprotegerin levels are influenced by voltage-dependent calcium channels Cav1.2. OPG can reduce the production of osteoclasts by inhibiting the differentiation of osteoclast precursors.

Guided bone regeneration (GBR) is relatively modern method and widely accepted among practitioners. The GBR technique refers to a surgical procedure which utilizes porous membranes as a mechanical barrier to create a secluded space around the defects to permit bone regeneration without the competition of other tissue [5]. Various Bone Grafting Materials (BGMs) are currently being used in regenerative procedures in orthopaedics and maxillofacial. Bone grafting materials are classified according to their origin, i.e., autografts (originating from the same individual), allografts (from the same species), xenografts (from another species) or alloplasts

(synthetic) [6]. The current study aimed at assessing the impacts of orthodontic force and type of bone substitute on nuclear factor-kB ligand and osteoprotegerin expression.

MATERIAL AND METHODS

The study was approved by the Ethics Committee of Fujian Medical University. All animal handling and surgical procedures were conducted according to the Institutional Review Board (IRB) guidelines for the use and care of laboratory animals.

Animal Subjects and Experimental Materials

This animal experimental study used six male beagle dogs aged 18 months with a mean weight of 11.8 Kg. Data were collected by GCF immunoassay analysis. Twenty-four alveolar bone defects were created by extending the first pre-molar extraction socket: The experimental defects were treated by guided bone regeneration (GBR) using synthetic β-TCP (Bio-lu Biomaterials Co., Ltd. Shanghai, China) or xenograft Bio-Oss® (Geistlich, Wolhusen, Switzerland) regeneration materials, whereas the control defects were left empty. Resorbable collagen membranes Bio-Gide® (Geistlich, Wolhusen, Switzerland) were used in both experimental and control groups.

The regeneration materials were equally allocated to the maxillary right and left (UR and UL) as well as to the mandibular right and left (LR and LL) defects by randomizing three pre-determined sets of defect managements to the six experimental animals as described in our previous work [7]. The set randomization also allowed for every GBR mode to be assigned to eight defects.

Surgical Procedure

Under general anaesthesia, the maxillary and mandibular first premolar extraction sockets were extended mesially to prepare standardized defects according to Machibya et al. [7]. Depending on the GBR mode allocation, the defects were filled with β-TCP or Bio Oss mixed with animal's blood collected during defect preparation. The mixture was packed into the artificial defects to the natural alveolar height level whereas; the control defects were left empty. The filled experimental and the empty control defects were all covered by resorbable collagen membranes Bio Gide® followed by wound closure using 3/0 nylon sutures which remained in the site for two weeks. According to the experiment protocol, the two months of OTM was commenced after two months of healing time.

A standardized clinical data sheet was used to collect clinical features of all defects for two weeks healing stage. Reported in a previous publication [7].

Gingival Crevicular Fluid (GCF) Collection

The gingival clavicular fluid samples were collected from all defects, one day before OTM and end of OTM (few minutes before termination of OTM). Prior to GCF collection, the animals were anaesthetized cleaned in the mouth and washed with normal saline. A methylcellulose paper strip was gently inserted in the gingival sulcus on the mesial aspect of second premolar and left in for 30 seconds. Afterwards, the paper strips were placed into Eppendorf tubes and preserved at -80°C. To quantify the GCF collected, the Eppendorf tubes with pater strips were weighed before and after sampling.

Orthodontic Appliance Design and Tooth Movement Assessment

The second premolar was moved to the mesial side by the application of a 150 g force as measured by a tension gauge (Aidebao, Leqing, China), using NiTi close coil spring (Ormco, Orang, County, CA, USA) (Figure 1).

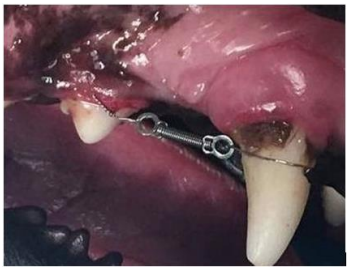

Figure 1: Appliance setup for orthodontic tooth movement (OTM).

The distance from the mesial-cervix of the second premolar to the distal-cervix of the canine was measured as previously described by Seifi et al. [8] at base line and end of OTM using a digital caliper (Guan lu, Guilin, China) with a precision of 0.01 mm.

Immunoassay Assessment of Gingival Crevicular Fluid

The OPG and RANKL expressions were examined by Enzyme linked immunosorbent assay in the GCF collected before and at the end of OTM to determine the impacts of regeneration materials and orthodontic force on the concentration of biomarkers in gingival crevicular fluid.

For immunoassay analysis, the samples were sent to Shanghai Biotechnologies, Inc. for protein extraction and immune assay process following the protocol previously described by Zhuang et al. [9].

RESULT

The OPG concentration registered at BL and end of OTM in experimental group was statistically significant different ($p < 0.05$). Nevertheless, the control group showed limited increase in OPG concentration at the end of OTM ($P = 0.09$) (Figure 2). Before OTM the control's OPG concentration was significantly higher than the Bio Oss group ($p < 0.05$). Although the β-TCP group registered higher concentration than both control and Bio Oss groups at the end of OTM, the difference fell short of statistical significance (Table 1).

Table 1: The mean and standard deviation of OPG and RANKL concentration in GCF according to different BRMs measured by ELISA test (ng/ml for OPG and pg/ml for RANKL).

Type of BRMs	OPG Concentration Mean (Std)		RANKL Concentration Mean (Std)	
	At BL	End of OTM	At BL	End of OTM
Bio Oss	0.222 (0.109)[a]	0.416 (0.167)	19.654(7.931)	30.586(20.565)[a]
β-TCP	0.300 (0.106)[ab]	0.498 (0.276)	19.232(9.866)	38.390(15.572)[b]
Control	0.331 (0.196)[a]	0.410 (0.231)	18.373(7.419)	26.992(16.074)[a]

[a-b]Means in a column without a common superscript letter differ ($P < 0.05$) as analysed by repeated measures ANOVA and the Tukey's test

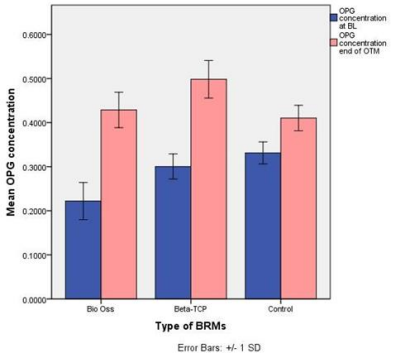

Figure 2: Bar chart displaying the OPG concentration in GCF according to different BRMs measured by ELISA test.

The RANKL concentrations registered at BL and end of OTM within groups were statistically significant different in all groups ($p < 0.01$) and the Control group had consistently lower concentration than experimental groups at BL and end of OTM (Table 1) (Figure 3). There was no RANKL concentration difference between groups at BL, but the β-TCP group had statistically higher concentration than control and Bio Oss groups at the end of OTM ($p < 0.05$) (Table 1).

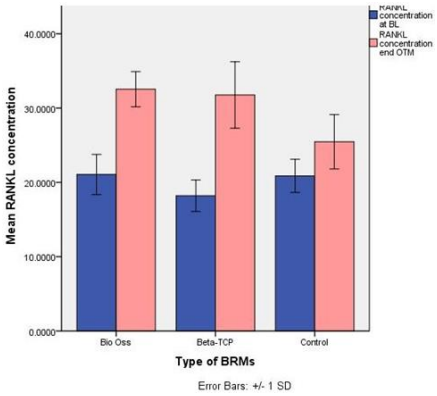

Figure 3: Bar chart displaying the RANKL concentration in GCF according to different BRMs measured by ELISA test.

Table 2: The mean and standard deviation of RANKL/OPG ratio in GCF according to different BRMs measured by ELISA test.

Type of BRMs	**RANKL/OPG Ratio Mean (Std)**	
	At BL	**End of OTM**
Bio Oss	164.121 (62.892)[a]	119.655 (161.406)[a]
β-TCP	57.766 (18.009)[a]	206.765 (238.578)[b]
Control	73.060 (54.926)[a]	148.137 (198.358)[ab]

[a-b]Means in a column without a common superscript letter differ ($P < 0.05$) as analysed by repeated measures ANOVA and the Tukey's test

Regarding RAKNL/OPG ratio; the Bio Oss registered significantly higher ratio than both control and β-TCP at BL. The RAKNL/OPG ratio at the end of OTM was statistically significantly higher than at BL for β-TCP ($p < 0.01$), but it decreased in Bio Oss group (Table 2). The RAKNL/OPG ratio difference between BL and end of OTM in Control group was statistically significant ($p < 0.05$) and was significantly higher in β-TCP compared to Bio Oss group at the end of OTM.

DISCUSSION

Several studies have shown significant association between OTM and cellular (osteoblasts and osteoclasts) remodelling activities coupled with OPG/RANK/RANKL signal pathway [10,11]. In the current study the differences observed in RANKL and OPG concentration is based on the type of BRMs as well as the orthodontic force (BL and end of OTM). There was statistically significant difference in OPG concentration between BL and end of OTM in experimental (Bio Oss and β-TCP), but the control group showed limited increase in OPG concentration at the end of OTM ($p = 0.09$) (Figure 2). The findings in the control group are in agreement with a study by Grant et al. [12] which reported insignificant OPG increases 4 hours after orthodontic force application at canine sites of both tension and compression and Otero et al [13] who found no statistically significant differences in OPG level on comparing force magnitudes and experimental teeth with those in the control teeth. The findings in the current study's experimental group differ from the control group (Table 1) (Figure 2) and those of previous studies [12,13]. The plausible explanation for the disparity is the difference in cellular and molecular components of regenerated bone and natural (non-regenerated) bone [13,10]. Shahoon et al. [14], in a histological study reported a gradual reduction of inflammation along with an increase in new bone formation in both Human Bone Matrix Gelatin (HBMG) and autograft groups on 7 days, 14 days, 28 days and 60 days after surgery, while autograft registered less giant inflammatory cells consistently. Although the actual molecular pathways involved in the remodelling is beyond the scope of this study, it is worth noting that the probable biological and chemical differences of bones regenerated by different BRMs could affect the OPG/RANK/RANKL signal pathway leading to the differences in OPG expression in the current study. The RANKL concentrations registered at BL and end of OTM were statistically significantly different in both experimental (Bio Oss and β-TCP) and control groups. Other studies [12,13,15] have observed increase in RANKL during resorption phases on pressure zone during OTM. Grant et al. [12] registered an increase in RANKL from day 7 days to 42nd days on pressure zone of teeth under orthodontic force. The control group in the current study had consistently lower concentration than experimental groups at BL and end of OTM (Table 1). Although the difference was not statistically significant, the observation may suggest lowered osteoclastic activities in control group than experimental group, particularly with β-TCP group. There was no RANKL concentration difference between groups at BL, but the β-TCP group registered statistically higher concentration than control and Bio Oss groups at the end of OTM. The presence of the material in the healing defect may have provided an ideal scaphoid for faster bone formation with adequate cellular component. Contrary to Bio Oss known for slow rate of material resorption [16,17], the β-TCP material might have undergone degradation and resorption at the end of OTM giving ideal biological environment for bone cells to respond effectively to the orthodontic mechanical force through OPG/RANK/RANKL signal pathway.

Many studies have demonstrated significant association between OTM and RANKL/OPG ratio, whereby the ratio tend to increase on pressure zone of teeth loaded with orthodontic forces [13,16,18,19]. The RAKNL/OPG

ratio in the current study increased for β-TCP and control groups but the Bio Oss group registered lower ratio at the end of OTM than BL. Bio Oss registered statistically higher ratio than both control and β-TCP at BL. Since osteoclasts are involved in bone remodelling during bone regeneration [18-20], the high RAKNL/OPG ratio observed in Bio Oss at BL may be due to active osteoclastic remodelling process as part of bone healing process even before OTM commencement. Some studies have suggested osteoclasts involvement in BRMs residual degradation during healing [21-24]. The low RAKNL/OPG ratio in β-TCP and control may be due to the lack of significant amount of residual material owed to the relatively faster rate of resorption for β-TCP and the lack of regeneration materials in the empty control group. The RAKNL/OPG ratio at the end of OTM was significantly higher in β-TCP compared to Bio Oss group. The difference was mainly because of increase in OPG concentration in Bio Oss group as well as significant increase in RANKL concentration in β-TCP at the end of OTM. The role of osteoblasts and osteoclasts in both bone healing and OTM is the probable explanation for the differences. The cellular and mineral component as well as morphological structure of regenerated bone varies with time and type of regeneration materials [13,25,26] which could affect the pattern of molecular expression of RANKL and OPG during OTM along regenerated bone defects. The rate of wound healing is reported to be significantly faster in dogs than in human, therefore the current study's findings cannot be directly inferred into clinical practice. Due to difference in bone turnover, the time correlating to the observed events can vary in clinical situation, although similar pattern of observations may be expected.

CONCLUSION

The orthodontic mechanical force as well as the type of BRMs significantly affected the RANKL and OPG concentration in GCF reflecting impact of biomechanical force and type of BRMs on biological activities at the site of bone regeneration.

CONFLICTS OF INTEREST

Authors declare no conflict of interest on any of the materials/products described in the manuscript.

REFERENCES

1. Oswal D, Sable RB, Patil AS et al. (2015) Levels of matrix metalloproteinase-7 and osteopontin in human gingival crevicular fluid during initial tooth movement. APOS Trends in Orthodontics 5: 77-82.
2. Nayak BN, Galil KA, Wiltshire W et al. (2013) Molecular biology of orthodontic tooth movement. Journal of Dentistry & Oral Health 1(101): 1-6.
3. Kurata H, Guillot PV, Chan J et al. (2007) Osterix induces osteogenic gene expression but not differentiation in primary human fetal mesenchymal stem cells. Tissue engineering 13(7): 1513-1523.
4. Patil A and Jayade VP (2006) Advances in biology of orthodontic tooth movement. A Review. Journal of Indian Orthodontic Society 39: 155-164.
5. Aronson J (1997) Current concepts review-limb-lengthening, skeletal reconstruction, and bone transport with the Ilizarov method. The Journal of Bone and Joint Surgery 79(8): 1243-1258.
6. Dimitriou R, Jones E, McGonagle D et al. (2011) Bone regeneration: Current concepts and future directions. BMC Medicine 9(1): 1-10.

7. Machibya FM, Zhuang Y, Guo W et al. (2018) Effects of bone regeneration materials and tooth movement timing on canine experimental orthodontic treatment. The Angle Orthodontist 88(2): 171-178.
8. Seifi M, Arayesh A, Shamloo N et al. (2015) Effect of nanocrystalline hydroxyapatite socket preservation on orthodontically induced inflammatory root resorption. Cell Journal 16(4): 514.
9. Zhuang Y, Machibya FM, Guo W et al. (2018) The effects of regenerative materials on biomarkers, clinical and radiological features of healing defects treated by guided bone regeneration. British Biomedical Bulletin 6(1): 312.
10. Theoleyre S, Wittrant Y, Tat SK et al. (2004) The molecular triad OPG/RANK/RANKL: Involvement in the orchestration of pathophysiological bone remodeling. Cytokine & growth factor reviews 15(6): 457-475.
11. Lin JM, Callon KE, Lin CQ et al. (2007) Alteration of bone cell function by RANKL and OPG in different in vitro models. European Journal of Clinical Investigation 37(5): 407-415.
12. Grant M, Wilson J, Rock P et al. (2013) Induction of cytokines, MMP9, TIMPs, RANKL and OPG during orthodontic tooth movement. European Journal of Orthodontics 35(5): 644-651.
13. Otero L, García DA, Wilches-Buitrago L (2016) Expression and presence of OPG and RANKL mRNA and protein in human periodontal ligament with orthodontic force. Gene Regulation and Systems Biology 10: GRSB-S35368.
14. Shahoon H, Azimi HR, Kianbakht C (2009) Comparison of the human bone matrix gelatin (HBMG) with autogenous bone graft in reconstruction of the parietal bone defects in rat: A histological and radiographic study. Journal of Dental Research, Dental Clinics, Dental Prospects 3(2): 37.
15. Arnez MFM, Ribeiro LSN, Barretto GD et al. (2017) RANK/RANKL/OPG expression in rapid maxillary expansion. Brazilian Dental Journal 28: 296-300.
16. Luvizuto ER, Queiroz TP, Margonar R et al. (2012) Osteoconductive properties of β-tricalcium phosphate matrix, polylactic and polyglycolic acid gel, and calcium phosphate cement in bone defects. Journal of Craniofacial Surgery 23(5): e430-e433.
17. Matsumoto MA, Caviquioli G, Biguetti CC et al. (2012) A novel bioactive vitroceramic presents similar biological responses as autogenous bone grafts. Journal of Materials Science: Materials in Medicine 23(6): 1447-1456.
18. Rody Jr WJ, Wijegunasinghe M, Wiltshire WA et al. (2014) Differences in the gingival crevicular fluid composition between adults and adolescents undergoing orthodontic treatment. The Angle Orthodontist 84(1): 120-126.
19. Kapoor P, Kharbanda OP, Monga N et al. (2014) Effect of orthodontic forces on cytokine and receptor levels in gingival crevicular fluid: A systematic review. Progress in Orthodontics 15(1): 1-21.
20. Devlin H and Sloan P (2002) Early bone healing events in the human extraction socket. International Journal of Oral and Maxillofacial Surgery 31(6): 641-645.
21. Blokhuis TJ, Termaat MF, den Boer FC ET AL. (2000) Properties of calcium phosphate ceramics in relation to their in vivo behavior. Journal of Trauma and Acute Care Surgery 48(1): 179.

22. Lorenz J, Kubesch A, Korzinskas T et al. (2015) TRAP-positive multinucleated giant cells are foreign body giant cells rather than osteoclasts: Results from a split-mouth study in humans. Journal of Oral Implantology 41(6): e257-e266.
23. Baslé MF, Chappard D, Grizon F et al. (1993) Osteoclastic resorption of Ca-P biomaterials implanted in rabbit bone. Calcified Tissue International 53(5): 348-356.
24. Ghanaati S, Barbeck M, Detsch R et al. (2012) The chemical composition of synthetic bone substitutes influences tissue reactions in vivo: histological and histomorphometrical analysis of the cellular inflammatory response to hydroxyapatite, beta-tricalcium phosphate and biphasic calcium phosphate ceramics. Biomedical Materials 7(1): 015005.
25. Rosa AL, Brentegani LG, Grandini SA (1995) Hydroxylapatite and tricalcium phosphate implants in the dental alveolus of rats. A histometric study. Brazilian Dental Journal 6(2): 103-109.
26. Bayat M, Momen-Heravi F, Marjani M et al. (2010) A comparison of bone reconstruction following application of bone matrix gelatin and autogenous bone grafts to alveolar defects: An animal study. Journal of Cranio-Maxillofacial Surgery 38(4): 288-292.