RESEARCH ARTICLE

The number of osteoblasts and osteoclasts in hypofunctional teeth during orthodontic tooth movement in rats [version 1; peer review: 1 approved, 1 approved with reservations]

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Abstract

Background: When moved orthodontically, hypofunctional teeth will have a decreased tooth movement rate compared to normal teeth. This study aimed to determine the number of osteoblasts in the tension side and the number of osteoclasts in the pressure side of the hypofunctional teeth during orthodontic tooth movement. **Method:** 18 male Wistar rats were given a palatal coil spring application on the maxillary incisors. Rats were divided into two groups, the orthodontic group with normal occlusion (NO) and hypofunctional occlusion (HO). The number of osteoblasts on the tension side and osteoclasts on the pressure side on days zero (D_0), five (D_5), and 10 (D_{10}) were tested with two-way ANOVA. Observations were made by hematoxylin eosin staining.

Result: The results showed that the number of osteoblasts on the tension side of the HO group was the same at the NO group ($p > 0.05$). The number of osteoblasts on the tension side in the NO and HO groups at D_5 was the same at D_{10} ($p = 0.99$), but significantly higher ($p = 0.002$), than D_0 . The number of osteoclasts on the pressure side in the HO group was significantly lower than the NO group ($p < 0.05$). The number of osteoclasts in the NO D_5 group was significantly higher than the other groups ($p < 0.05$).

Conclusions: The number of osteoblasts on the tension side was not affected by the hypofunctional state but decreased the number of osteoclasts on the pressure side during orthodontic tooth movement.

Keywords

Tooth movement, osteoblast, osteoclast, hypofunctional

Open Peer Review

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06 Jul 2021	view	view

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Any reports and responses or comments on the article can be found at the end of the article.

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Introduction

Tooth movement in orthodontic treatment is a biological response to mechanical forces characterized by remodeling processes in dental and paradental tissue, including pulp tissue, periodontal ligaments, alveolar bone, and gingiva.¹ Osteoblasts, osteoclasts, and osteocytes play an essential role in bone remodeling in orthodontic tooth movement.²

Clinicians often encounter cases which need to move the teeth that functionally never have occlusal pressure or hypofunctional teeth, such as open bite, ectopic canine, linguioversion and bucoverversion teeth.³ Open bite malocclusion occurs when maxillary and mandibular teeth are not in contact.⁴

Hypofunctional teeth cause atrophic changes in the periodontal ligament, a decrease in the number of periodontal fibers and blood vessels, and the periodontal space's narrowing. Periodontal space's narrowing occurs due to the apposition of the alveolar bone by an increase in Transforming Growth Factor β (TGF β), causing tooth elongation.⁵ Changes in the paradental structure of hypofunctional teeth cause different reactions when orthodontically moved than normal teeth, especially in periodontal ligament tissue. Hypofunctional teeth when orthodontically moved have less heparan sulfate proteoglycan exposure, which plays a role in the osteoclastic activity, compared to normal teeth.⁵ Expression of Vascular Endothelial Growth Factor (VEGF) in hypofunctional teeth also decreases during orthodontic movement leading to vascular constriction and endothelial cell apoptosis. The expression of VEGF has an important role in the resorption and apposition processes of alveolar bone because it affects the proliferation and differentiation of osteoblasts and osteoclasts in vitro.⁶ Research on the number of osteoblasts on the tension side and osteoclasts on the pressure side in hypofunctional teeth during orthodontic movement has never been done before. This study aimed to determine the number of osteoblasts on the tension side and osteoclasts on the pressure side on hypofunctional teeth, respectively, during orthodontic tooth movement.

Wistar rats are considered a good research model for this study into orthodontic tooth movement because rats are cheap, making them easy to use as a large quantity sample, and the histological profiles of rats are easy to compare, especially in incisor teeth. Incisor teeth of Wistar rats have a gingival structure that almost resembles humans', and is easy to install the orthodontic appliance into.

Methods

Ethical considerations

All experimental procedures were performed according to the Institutional Animal Care and Usage Committee (ARRIVE guidelines). The Ethical Clearance was approved by Ethical Committee of the Faculty of Dentistry of Universitas Gadjah Mada Yogyakarta, Indonesia, with Ethical Clearance number 00288/KKEP/FKG-UGM/EC/2019. All procedures involving rats were carried out with consideration to eliminate any suffering in the rats by using anesthetic drugs and euthanasia procedures during rats' tissue collection.

Animals

This study used 18 five-month-old, male, healthy rats weighing $\pm$ 400 grams, which had never been used in any procedures before. Rats adapted beforehand for seven days on a standard diet, including pellets. Rats were placed in cages at room temperature, which was 26C. Inclusion criteria related to body weight, sex, age, and health condition of the rats. Exclusion criteria included any technical issues that could disrupt orthodontic tooth movement, such as trapped bonding inside palatal coil.

Experimental animals were divided into two groups: the normal occlusion (NO) and the hypofunctional occlusion group (HO), both were moved orthodontically. This study was done without a control group in order to examine orthodontic tooth movement with and without occlusion over a period of time. In the hypofunctional group, the mandibular left incisors were cut to the gingival margin level every two days to obtain consistent spacing throughout the study. The sample size was determined using the Federer formula. Each group consisted of three rats with three groups of observation days: day zero (D0), day five (D5), and day 10 (D10). Rats were allocated to their groups using a simple randomization method: each rat was labelled, and a blindfolded researcher drew corresponding labels from a hat for each group. Researchers were aware of which group was which during the experiment.

Procedures

Animals were anesthetized using 10% ketamine 35 mg/kg and 2% xylazine 5 mg/kg intramuscularly during spring installation and reduction of left lower incisor. The upper incisors were separated using a customized palatal coil spring of 0.012 mm stainless steel wire (Ortho Prime Inc. USA: A 85021201; orthoshape SS 0,012") connected to two metal bands (Dentaurum) with the arm length is 5 mm and the coil diameter is 2 mm. The customized coil spring was deflected for 3.4 mm to deliver an orthodontic force of 17.5 cN per upper incisor before being installed.⁷ The palatal coil spring was cemented using GIC Fuji IX, as shown in [Figure 1](#). Then the left lower incisor was cut.

Figure 1. Installation of a palatal coil spring on rat incisors accompanied by reduction of lower incisors.

All experimental animals in day zero, day five, and day 10 groups were euthanized using an overdose solution of ketamine and xylazine (lethal dose: ketamine (KEPRO.BV production), 300 mg/kg BW and brand xylazine (Xyla) 30 mg/kg BW) intraperitoneally. Cross sections were taken on alveolar crest region of the upper incisor, shown in **Figure 2**. The number of osteoblasts were counted on the tension side and osteoclasts were counted on the pressure side using hematoxylin eosin staining and observed using an optical microscope (Olympus) with 400 times magnification in three fields of view every slide. Osteoblast cells appear cuboidal or columnar, purple, and single-nucleated. Osteoclast cells appear multinucleated with random boundaries, and purple in the resorption lacunae.

Figure 2. Alveolar crest region of the upper incisor where cross-section was taken shown in the black rectangle.

Statistical analysis

The program used to perform statistical analysis was SPSS version 17.0 for Windows. Cohen's Kappa test value from two observers showed more than 0.50, which means there was good agreement between the two observers. All data were normally distributed and homogeneous. The research data were then analyzed using the two-way ANOVA test followed by the Post Hoc test, Multiple Comparison (LSD). The confidence level used in this study is 95%.

Results

The results in Table 1 show that the number of osteoblasts on the tension side of the hypofunctional group is higher than the normal group, but the difference is not significant ($p = 0.187$). The number of osteoblasts in the normal occlusal contact group (NO) increased significantly on day five and continued to increase until day 10, as seen in Table 2, in contrast to the hypofunctional group, which increased until day five but slightly decreased on day 10.

Table 1. Mean of osteoblasts on the tension side (cells/field).

Group	Mean $\pm$ SD		
	D ₀	D ₅	D ₁₀
NO	60.66 $\pm$ 9.50	112.33 $\pm$ 14.84	116 $\pm$ 18.24
HO	80.33 $\pm$ 9.50	124 $\pm$ 19.15	123 $\pm$ 38.93

Abbreviations: SD, standard deviations; NO, normal occlusion group; HO, hypofunctional occlusions group; D₀, day zero; D₅, day five; D₁₀, day 10.

Table 2. Post Hoc LSD of osteoblasts on the tension side in each observation day.

Group	D ₀	D ₅	D ₁₀
D ₀	-	0.002*	0.002*
D ₅	-	-	0.990
D ₁₀	-	-	-

Abbreviations: D₀, day zero; D₅, day five; D₁₀, day 10.
Note: Tested by two-way ANOVA and post Hoc LSD test.

Figure 3. Osteoblast cells (arrow) of normal occlusion group (A) and osteoblast cells of hypofunctional group (B) in the tension side during orthodontic tooth movement. AB: Alveolar Bone, PDL: Periodontal Ligament.

The highest number of osteoblasts on the tension side was seen in the hypofunctional group on day five. The lowest number of osteoblasts on the tension side was seen in the orthodontic tooth movement group with normal occlusion on day zero.

The number of osteoblasts in the normal occlusion group (A) and the number of osteoblasts in the hypofunctional group (B) in the tension side during tooth movement is shown in Figure 3. Figure 4 showed the number of osteoclasts in the normal occlusion (C) and the hypofunctional groups (D) in the pressure side during orthodontic treatment.

The results in Table 3 showed that the number of osteoclasts in the hypofunctional group was significantly lower than the normal occlusion group on each day of observation ($p = 0.014$). The number of osteoclasts on the pressure side during orthodontic tooth movement in the normal occlusion group began to significantly increase until day five, as seen in

Figure 4. Osteoclast cells (arrows) of normal occlusion group (C) and osteoclast cells of hypofunctional group (D) in pressure side during orthodontic tooth movement. AB: Alveolar Bone, PDL: Periodontal Ligament.

Table 3. Mean of osteoclasts on the pressure side (cells/field).

Group	Mean $\pm$ SD		
	D ₀	D ₅	D ₁₀
NO	1.33 $\pm$ 1.52	4 $\pm$ 2	0.67 $\pm$ 0.577
HO	0	1.33 $\pm$ 1.15	0

Abbreviations: SD, standard deviations; NO, normal occlusion group; HO, hypofunctional occlusions group; D₀, day zero; D₅, day five; D₁₀, day 10.

Table 4. Post Hoc LSD of osteoclasts on the pressure side in each observation day.

Group	D ₀	D ₅	D ₁₀
D ₀	-	0.011*	0.626
D ₅	-	-	0.004*
D ₁₀	-	-	-

Abbreviations: D₀, day zero; D₅, day five; D₁₀, day 10.

Note: Tested by two-way ANOVA and post Hoc LSD test.

Table 4, then decreased on day 10. This pattern was the same as in the hypofunctional group, which increased until day five, then decreased on day 10. The highest number of osteoclasts on the pressure side was seen in the normal occlusion group on day five.

Discussion

The study showed that the number of osteoblasts in the normal occlusion group had increased significantly on day five and then showed no significant difference until day 10, as seen in **Table 2**. This result was in line with Herniyati's⁸ research, which stated that the formation of preosteoblasts from mesenchymal cells had occurred 10 hours after applying force, followed by the differentiation of osteoblasts 40-48 hours later. The maximum number of osteoclasts was reached on the 6th day of orthodontic tooth movement.⁹ This osteoblast differentiation and proliferation lasted up to 10 days.¹⁰

The increasing number of osteoblasts on the tension side during 10 days of observation occurs because in the early phase of orthodontic tooth movement there will be an acute inflammatory response characterized by periodontal tissue vasodilation and prostaglandin secretion and growth factors such as TGF β .¹ TGF β is also produced by fibroblasts on the tension side. TGF β is an important factor in osteoblastogenesis and bone formation by recruiting osteoblast progenitors and stimulating the differentiation of bone matrix. An increase in TGF β will increase osteoblast proliferation on the tension side.¹¹ This acute inflammatory response will lead to an increasing number of osteoblasts in the early phase. One to two days later, the acute phase of inflammation is replaced by a chronic inflammatory process that is more proliferative, involving fibroblasts, endothelial cells, and osteoblasts.¹

The number of osteoblasts on the tension side during orthodontic movement of teeth with normal occlusion is influenced by several growth factors that are sensitive to mechanical stimuli, such as the expression of TGF β , VEGF, Fibroblast Growth Factor (FGF), and Insulin-like Growth Factor (IGF). The increase in growth factor on the tension side will cause an increase in the number of osteoblasts. Hypofunctional teeth, without orthodontic force, will experience an increase in TGF β expression, which simultaneously decreases VEGF, IGF, and FGF expression in the periodontal tissue.^{5,6,12,13} Transforming Growth Factor β has a role in stimulating osteoblast differentiation and osteoclast apoptosis.⁵ Decreased FGF will lead to osteoblast differentiation in hypofunctional teeth because FGF works to inhibit osteoblast differentiation.¹³ The decrease in IGF causes a decrease in osteoblast proliferation because IGF is dominant in providing osteogenic effects.¹⁴

Teeth that are hypofunctional when moved orthodontically will tend to experience decreased VEGF expression on both the tension and pressure sides.⁶ Decreased VEGF expression will cause apoptosis of endothelial cells, causing vascular constriction and decreased permeability. This will reduce the migration of osteoblasts on the tension side.⁶ Increased TGF and decreased FGF in hypofunctional teeth will increase osteoblasts.

The results showed the number of osteoblasts on the tension side of the hypofunctional teeth was the same as normal teeth during orthodontic movement ($p > 0.05$). This was possible because before orthodontic movement there was an increase in osteoblasts due to the interaction of increasing TGF β and decreasing FGF and IGF, but simultaneously when hypofunctional teeth were given orthodontic force, there was a decrease in VEGF which tended to decrease osteoblast differentiation and migration, so that the number of osteoblasts became the same as the normal group. This needs further research.

The results showed the number of osteoclasts on the pressure side of the normal group began to increase on the first day after the installation of a palatal coil spring and continued increasing until the fifth day, then decreased. on day 10. This result was almost the same as in the hypofunctional group, which increased up to day five, then decreased on day 10. This result is in line with the study by Miyoshi¹⁵ which states that orthodontic movements immediately after force application are almost absent in osteoclasts. After the third day of mechanical strength application, several osteoclasts appeared. The maximum number of osteoclasts was reached on day six of orthodontic tooth movement.⁹ The increase in osteoclasts on day three was in line with the increase in VEGF expression, which also increased sharply.¹⁶

An increasing number of osteoclasts occur because, in the early phase, the mechanical stress in the compression area will stimulate mechanoreceptors on osteocytes and cause changes in flow and blood vessels, causing tissue hypoxia that activates VEGF.¹⁷ VEGF plays an essential role in the angiogenesis process in the area of hyalinization.¹⁶ VEGF also plays a role in vascular permeability and activates endothelial cells. Active endothelial cells in the area of compression will cause chemoattraction of acute inflammatory cells such as leukocytes, monocytes, and macrophages. Leukocytes will stimulate prostaglandins and macrophage-colony stimulating factor (M-CSF). Increased prostaglandins in the area of compression will stimulate osteoblast differentiation and receptor activator of nuclear factor-kappa B ligand (RANKL)

expression, whereas M-CSF can induce osteoclast differentiation by attaching to the c-Fms receptor on monocytic lineage cells. RANKL and M-CSF play an essential role in the process of osteoclast differentiation and bone resorption.¹⁷

The number of osteoclasts on the pressure side in the hypofunctional group was smaller than the normal occlusion group on each observation day. This result was probably because VEGF expression in hypofunctional teeth decreases during orthodontic movement leading to vascular constriction and endothelial cell apoptosis.⁶ Endothelial cell apoptosis will cause decreased osteoclast differentiation and bone resorption.¹⁷ The decrease in VEGF will also cause a decrease in vascular permeability so that it will significantly imply a decrease in the number of osteoclasts.¹⁸

Orthodontic tooth movement involves osteoblastic activity on the tension side and osteoclastic activity on the pressure side.¹ The decrease in the number of osteoclasts on the pressure side in the orthodontic tooth movement of this hypofunctional tooth group suggests a possible decrease in the rate of orthodontic tooth movement. This is in line with the research of Usami-Fujita⁶ which states that there is a decrease in the rate of orthodontic movement in hypofunctional teeth.

Conclusion

In conclusion, the number of osteoblasts on the tension side was not affected by the hypofunctional condition but decreased the number of osteoclasts on the pressure side during orthodontic tooth movement. The number of osteoclasts in hypofunctional teeth is lower compared to the normal group during orthodontic tooth movement. It is possible that this is because of the decrease in VEGF and heparan sulfate proteoglycan.

Data availability

Underlying data

Figshare: The Number of Osteoclast and Osteoblast in Hypofunctional Teeth during orthodontic tooth movement. <https://doi.org/10.6084/m9.figshare.14515740.v8>.

This project contains the following underlying data:

- osteoclasts.xlsx
- osteoblasts.xlsx
- table of statistic analysis.docx
- Figure 1. jpg
- Figure 2. jpg
- Figure 3 A.jpg
- Figure 3 B.jpg
- Figure 4 C.jpg
- Figure 4 D.jpg

Reporting guidelines

Figshare: ARRIVE Checklist, Maulani et al. <https://doi.org/10.6084/m9.figshare.14515740.v8>.

Data are available under the terms of the [Creative Commons Zero “No rights reserved” data waiver](#) (CC0 1.0 Public domain dedication).

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[Publisher Full Text](#)

Open Peer Review

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Masaru Yamaguchi

Ginza Orthodontic Clinic, Tokyo, Japan

This paper describes that the number of osteoblasts and osteoclasts in hypofunctional teeth during orthodontic tooth movement in rats.

Generally, this manuscript is interesting. However, there are some concerns as presented and some of these are discussed below.

Major Comments:

1. Was the force in this study optimal? The optimal force for molar tooth movement in rat may be 10g. The 25cN may induce an undermining bone resorption. In incisor, 17.5 cN force may be strong.
2. How much was the amount of tooth movement? Please add the results of them.
3. I think that research on Hypofunction is generally more suitable for molars, but how about it?
4. The authors concluded that the number of osteoblasts on the tension side was not affected by the hypofunctional state but decreased the number of osteoclasts on the pressure side during orthodontic tooth movement. However, this statement seems inconsistent. Did the number of osteoblasts on the tension side in hypofunction group also decreased?
5. I think that immunostaining such as TGF-b, VEGF, FGF, and IGF will be a better paper.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Dental, Orthodontics, bone metabolism, inflammation

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 08 Apr 2022

Adibah Maulani, Faculty of Dentistry, Gadjah Mada University, Sleman, Indonesia

Dear Dr. Masaru Yamaguchi

Thank you for your kind assistance in reviewing our manuscript and providing us with valuable advices. Please allow us to comment as follows :

Was the force in this study optimal? The optimal force for molar tooth movement in rat may be 10g. The 25cN may induce an undermining bone resorption. In incisor, 17.5 cN force may be strong.

Thank you very much for your insight. The force in this study was moderate force and already used in previous study about the rate of orthodontic tooth movement in rat incisor.

How much was the amount of tooth movement? Please add the results of them.

Thank you very much for your suggestion. We already revised in new version by adding the rate of orthodontic tooth movement of this study.

I think that research on Hypofunction is generally more suitable for molars, but how about it?

Hypofunctional research is usually done on molar because the hypofunctional state is easier to obtain by removing the upper molar teeth of rats. However, in this study, we chose incisors in order to facilitate the installation of orthodontic appliances and the anatomical structure of the periodontal tissue of the rat incisor was almost similar to humans. The hypofunctional state in this study was obtained by cutting the lower incisors of rats every two days.

The authors concluded that the number of osteoblasts on the tension side was not affected by the hypofunctional state but decreased the number of osteoclasts on the pressure side during orthodontic tooth movement. However, this statement seems inconsistent. Did the number of osteoblasts on the tension side in hypofunction group also decreased?

The number of osteoblast on the tension side in hypofunction group was actually increase, but not significant. Because it was not significant, we conclude that the number of osteoblast on tension side was not effected.

I think that immunostaining such as TGF- β , VEGF, FGF, and IGF will be a better paper.

Thank you very much for your suggestion. We would continue the study to know deeper using immunostaining.

Competing Interests: No competing interests were disclosed.

Reviewer Report 15 July 2021

<https://doi.org/10.5256/f1000research.57143.r89164>

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Erliera Sufarnap

Department of Orthodontics, Universitas Sumatera Utara Fakultas Kedokteran Gigi, Medan, Indonesia

Please allow me to congratulate the authors to perform this research study which I found quite interesting and useful as a reference for further study in the orthodontics field. We have been reviewing the manuscript.

However, I may require some clarifications on the following issues. My reviews recommend reconsideration to have some minor revisions which I will addressing the inquiries below.

General comment:

The paper is well organized and easy to follow. It also well-written and well-structured but unfortunately there were had some typos, inconsistent of using words or abbreviation, had an ambiguous comprehension in reporting the analysis results, and many statement didn't follow with references. I suggest the revision of the English grammar structures by an expert and also to the statistician to interpret the result.

A. Abstract:

- Background:

- “When moved orthodontically, hypofunctional teeth will have a decreased tooth movement rate compared to normal teeth” - This background already described the results indirectly which can correlated to lower osteoclast production and increased osteoblast. Please find another reason as a research gap which doesn't comprehend to the OTM, maybe from the periodontal point of view.

- Results:

1. Please describe the exact numbers of the p-value of each osteoblast and osteoclast, and compare mean between groups as the result.
2. Osteoblast: The post hoc analysis which had been analysed to “compare” between each time points (internally) for both groups and please further describe with statistically words and maintain the chronological time points, i.e., significantly increased at D0-D5, D0-D10 and not significantly changed at D5-D10.

B. Introduction:

1. At the last chapter, the research gap and the objective of the study clearly mentioned but We couldn't see any hypothesis described in the introduction.
2. Describe the reference of why the animal model being used

C. M&M:**1. Animals:**

- Please explain scientifically with reference the rationale to choose the male Wistar rats. The hypofunctional teeth could be happened to all genders.
- “...without a control group”; In my opinion, the NO already intervened as a control group.
- The teeth were cut to the gingival margin level every 2 days. Explain about the teeth and pulp anatomical condition. Please also mention why the teeth were cut every 2 days.

2. Procedures:

- Figure 1: We couldn't see properly the palatal coil spring.
- Microscope (Olympus); please provide the type of the Olympus microscope.
- Please provide the reference which were mentioned for the osteoblast and osteoclast characteristics

3. Statistical analysis:

- Who were the two observers? Please mention in the manuscript.

D. Results:

1. The post hoc analysis were compared the differences between each time point for both HO

and NO groups. Please describe those results based on the data, i.e, at D0 to D5, D0 to D10 and D5 to D10.

2. Please consistent of using "the group's name"; with or without abbreviation. As if it will choose without abbreviation please further consistent of using complete group's name. For some journal, they would prefer mention with abbreviation though.

E. Discussion:

1. At the first sentence for osteoblast discussion it discussed about time interval analysis, the author described only NO group unfortunately from Table 2 only had 1 result, did it mean that the statistic aimed to compare between time for both groups together? It supposed that the HO and NO increased together significantly at D0-D5 and D0-D10. Please, it would be more satisfy as if the author would ask the statistician whether the post hoc analysis results addressed for each group or for both group.
2. The first paragraph described all about osteoblast, but at the second last sentence it described about osteoclast. It has been incompatible discussion.
3. The second and third paragraphs describes slightly about Growth factors. Please describes first from the general (all GF) and then followed to each GF discussion.
4. Typos found in the TGF abbreviation supposed to be TGFb.
5. Some discussion for several paragraph didn't have references.
6. We couldn't find any study's limitation. As if the study were flawless it would be accepted.

F. Conclusion:

1. Please conclude the study based on the results.
2. Please be consistent in using the groups name.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: cellulars in the orthodontic tooth movement research

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 08 Apr 2022

Adibah Maulani, Faculty of Dentistry, Gadjah Mada University, Sleman, Indonesia

Dear Dr. Erliera Sufarnap

Thank you for your kind assistance in reviewing our manuscript and providing us with valuable advices. Please allow us to comment as follows :

Background:

“When moved orthodontically, hypofunctional teeth will have a decreased tooth movement rate compared to normal teeth” - This background already described the results indirectly which can correlated to lower osteoclast production and increased osteoblast. Please find another reason as a research gap which doesn’t comprehend to the OTM, maybe from the periodontal point of view.

Hypofunctional teeth would have less VEGF expression and decreased heparan sulfate proteoglycan production during orthodontic tooth movement. The study about periodontal structure of hypofunctional teeth that moved orthodontically is never been done before.

Results:

Please describe the exact numbers of the p-value of each osteoblast and osteoclast, and compare mean between groups as the result.

p-value of osteoblast between NO and HO group is 0.187

p-value of osteoclast between NO and HO group is 0.014

Osteoblast: The post hoc analysis which had been analysed to “compare” between each time points (internally) for both groups and please further describe with statistically words and maintain the chronological time points, i.e., significantly increased at D0-D5, D0-D10 and not significantly changed at D5-D10.

Osteoblast significantly increased at D0 to D5 with p-value 0.002, and so is at D0 to D10. However, at D5 to D10, the number of osteoblast is not significantly different, with p-value 0.99.

B. Introduction:

At the last chapter, the research gap and the objective of the study clearly mentioned but We couldn’t see any hypothesis described in the introduction.

Hypotheses of the study are:

- The number of osteoblasts on the tension side of the hypofunctional teeth is higher than normal teeth during orthodontic tooth movement.
- There is an increase in the number of osteoblasts and osteoclast of the hypofunctional teeth during 5 and 10 days of orthodontic tooth movement.
- The number of osteoclasts on the pressure side of the alveolar bone in hypofunctional teeth lower than normal teeth during orthodontic tooth movement.

Describe the reference of why the animal model being used

This study used animal model because we would like to see histologically during orthodontic tooth movement in hypofunctional teeth. The histological profiles of rats are easy to compare, especially in incisor teeth. Incisor teeth of Wistar rats have a gingival structure that almost resembles humans (Ren *et al.*, 2004). Thank you for your kind assistances. We would complete the references in new version of the article.

C. M&M:

1. Animals:

Please explain scientifically with reference the rationale to choose the male Wistar rats. The hypofunctional teeth could be happened to all genders.

The reason why we choose male Wistar rats is because we avoid hormonal influences in female Wistar rats during orthodontic tooth movement.

“...without a control group”; In my opinion, the NO already intervened as a control group.

Thank you very much for your insight.

The teeth were cut to the gingival margin level every 2 days. Explain about the teeth and pulp anatomical condition. Please also mention why the teeth were cut every 2 days.

Wistar rats is a rodent, so the incisor teeth will erupt continuously. To maintain the space between maxillary incisor teeth and mandible incisor teeth, the teeth were cut to the gingival margin every 2 days. The teeth and pulp was still in good condition even after being cut every 2 days. This method was done based on previous study by Silva and Merzel (2004).

2. Procedures:

Figure 1: We couldn't see properly the palatal coil spring.

Thank you very much for your suggestion, I have revised it in recent article.

Microscope (Olympus); please provide the type of the Olympus microscope.

Olympus CX-22 (Olympus, Germany).

Please provide the reference which were mentioned for the osteoblast and osteoclast characteristics

Osteoblast characteristic was based on previous study by Alhasyimi *et al.*, (2018) which mentioned that osteoblast is cuboid cell with single and deep-blue nucleus. Osteoclast cells appear multinucleated with random boundaries, and purple in the resorption lacunae

(Flores-silva *et al.*, 2015). Thank you for your kind assistances. We would complete the references in new version of the article.

3. Statistical analysis:

Who were the two observers? Please mention in the manuscript.

The two observer were two-trained person who performed the measurement of osteoblast and osteoclast cell histologically. They were blinded to the applied sample. Thank you very much for your suggestion, I have revised it in recent article.

D. Results:

The post hoc analysis were compared the differences between each time point for both HO and NO groups. Please describe those results based on the data, i.e, at D0 to D5, D0 to D10 and D5 to D10.

Osteoblast significantly increased at D0 to D5 with p-value 0.002, and so is at D0 to D10. However, at D5 to D10, the number of osteoblast was not significantly different, with p-value 0.99.

Osteoclast significantly increased at D0 to D5 with p-value 0.011. However, osteoclast significantly decreased at D5 to D10 with p-value 0.004. The number of osteoclast between D0 to D10 was not significantly different, with p-value 0.626.

Please consistent of using "the group's name"; with or without abbreviation. As if it will choose without abbreviation please further consistent of using complete group's name. For some journal, they would prefer mention with abbreviation though.

Thank you very much for your suggestion, I have revised it in recent article.

E. Discussion:

At the first sentence for osteoblast discussion it discussed about time interval analysis, the author described only NO group unfortunately from Table 2 only had 1 result, did it mean that the statistic aimed to compare between time for both groups together? It supposed that the HO and NO increased together significantly at D0-D5 and D0-D10. Please, it would be more satisfy as if the author would ask the statistician whether the post hoc analysis results addressed for each group or for both group.

Thank you very much for your suggestion. The result between HO and NO group is not significantly different. Increasing number of osteoblast from D0 to D5 and then D10 on HO group has the same pattern with NO group, so the Post Hoc analysis is aimed to compare between time for both group together.

The first paragraph described all about osteoblast, but at the second last sentence it described about osteoclast. It has been incompatible discussion.

Thank you for your kind assistance, I apologize for the typo. I have revised it in recent article.

The second and third paragraphs describes slightly about Growth factors. Please describes first from the general (all GF) and then followed to each GF discussion.

Growth factors are proteins that attach to receptors on the cell surface, thereby activating a signal transduction, and subsequently affecting cell proliferation, differentiation, and

apoptosis. Most growth factors have a specific effect on certain cell types on the process of proliferation and differentiation. For example, Fibroblast Growth Factor (FGF), Transforming Growth Factor (TGF)- β , and Insulin-like Growth Factor (IGFs), required for growth of bone, muscle, and other cells (Stone *et al.*, 2022).

TGF- β (Transforming Growth Factor β) is growth factor that can affect bone metabolism. This mediator regulates bone remodeling by modulating osteoblasts and osteoclasts (Fox and Lovibond, 2005).

Transforming Growth Factor has the role of stimulating differentiation osteoblasts and osteoclast apoptosis (Itohiya *et al.*, 2016).

FGF (Fibroblast Growth Factor) is a representative growth factor which has shown the potential effects on the repair and regeneration of tissues (Moya *et al.*, 2010).

Basic fibroblast growth factor (bFGF) also involved in stimulation of osteoblasts and osteoclasts (Seifi *et al.*, 2013).

IGF, induced by growth hormone and parathyroid hormone, is reproduced by osteoblast, chondrocytes and other bone cells, and plays an essential part as a local modulator in cell differentiation and proliferation in either an autocrine or paracrine manner. Igf-I stimulates not only osteoblast proliferation but also osteoclast formation (Tokimasa *et al.*, 2003).

VEGF (Vascular Endothelial Growth Factor) is an essential mediator during the process of angiogenesis, bone remodelling by stimulating osteoblast differentiation, and osteoclastic recruitment (Yang *et al.*, 2012)..

Typos found in the TGF abbreviation supposed to be TGF β .

Thank you very much for your suggestion, we have revised the typos in new version of the article.

Some discussion for several paragraph didn't have references.

Thank you for your kind assistances. We would complete the references in new version of the article.

We couldn't find any study's limitation. As if the study were flawless it would be accepted.

Thank you for your kind suggestions, this study's limitations including days of observations which was limited for 10 days. Further study should provide longer time, at least 14 days up to 21 days, to observe further effect of hypofunctional condition in the number of osteoblast and osteoclast cell during orthodontic tooth movement.

F. Conclusion:

Please conclude the study based on the results.

The number of osteoblasts on the tension side was not affected by the hypofunctional condition but the number of osteoclasts on the pressure side was lower during orthodontic tooth movement under hypofunctional condition.

Please be consistent in using the groups name

Thank you very much for your suggestion, we have revised the groups name recent article.

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Competing Interests: No competing interests were disclosed.

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