© 2021 Journal of Pharmacy & Pharmacognosy Research, 9 (4), 446-453, 2021
ISSN 0719-4250
http://jppres.com/jppres

Original Article

The increased basic fibroblast growth factor expression and osteoblasts number post *Bifidobacterium bifidum* probiotic supplementation during orthodontic tooth movement in Wistar rats

[Aumento de la expresión del factor de crecimiento básico de fibroblastos y el número de osteoblastos después de la suplementación con probióticos de *Bifidobacterium bifidum* durante el movimiento dental de ortodoncia en ratas Wistar]

Ari Triwardhani[1]*, Citra Anggitia[1], I Gusti Aju Wahju Ardani[1], Alexander Patera Nugraha[1], Wibi Riawan[2]

[1]Departement of Orthodontic, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.
[2]Department of Biomolecular Biochemistry, Faculty of Medicine, Universitas Brawijaya, Surabaya, Indonesia.
*E-mail: ari-t@fkg.unair.ac.id

Abstract

Context: *Bifidobacterium bifidum* as a beneficial probiotic may regulate the inflammation and induce the bone remodeling by enhancing osteoblasts and basic fibroblast growth factor (bFGF) expression in the tension side of alveolar bone during orthodontic tooth movement (OTM).

Aims: To evaluate the expression of bFGF and the number of osteoblasts during OTM after *B. bifidum* probiotic supplementation in male Wistar rats.

Methods: Wistar rats (n = 42) were divided into 6 groups (n = 7) accordingly as follow: OTM and phosphate buffer saline (PBS) for 3 days (K3); OTM and PBS for 7 days (K7); OTM and PBS for 14 (K14); OTM and *B. bifidum* for 3 days (P3); OTM and *B. bifidum* for 7 days (P7); OTM and *B. bifidum* for 14 days (P14). OTM was established by NiTi close coil spring with 10 g force-placed between the first incisor and the first maxillary molar of Wistar rat. The samples were then terminated on days 3, 7, and 14. The maxillary tissue was isolated for the immunohistochemical examination and hematoxylin-eosin staining. All data were analyzed by using an independent t-test ($p<0.05$), which was implemented based on Kolmogorov-Smirnov and Levene's test ($p>0,05$).

Results: The highest bFGF expressions and osteoblast numbers were found in Group P14. There were significant differences in bFGF expression and osteoblast numbers in the tension side of alveolar bone during OTM between the control and treatment groups ($p<0.05$).

Conclusions: The post supplementation of *B. bifidum* shows the enhancement of bFGF expression, and osteoblast number in the tension side of alveolar bone during OTM determined immunohistochemically.

Keywords: basic fibroblast growth factor; *Bifidobacterium bifidum*; orthodontic tooth movement; osteoblast.

Resumen

Contexto: *Bifidobacterium bifidum*, como probiótico beneficioso, puede regular la inflamación e inducir la remodelación ósea al mejorar la expresión de los osteoblastos y el factor de crecimiento básico de fibroblastos (bFGF) en el lado de tensión del hueso alveolar durante el movimiento dental de ortodoncia (OTM).

Objetivos: Evaluar la expresión de bFGF y el número de osteoblastos durante la OTM después de la suplementación con probióticos de *B. bifidum* en ratas Wistar macho.

Métodos: Se dividieron ratas Wistar (n = 42) en 6 grupos (n = 7) de la siguiente manera: OTM y solución salina tamponada con fosfato (PBS) durante 3 días (K3); OTM y PBS durante 7 días (K7); OTM y PBS para 14 (K14); OTM y *B. bifidum* durante 3 días (P3); OTM y *B. bifidum* durante 7 días (P7); OTM y *B. bifidum* durante 14 días (P14). El OTM se estableció mediante un resorte helicoidal cerrado de NiTi con una fuerza de 10 g colocada entre el primer incisivo y el primer molar superior de la rata Wistar. A continuación, se terminaron las muestras los días 3, 7 y 14. Se aisló el tejido maxilar para el examen inmunohistoquímico y la tinción con hematoxilina eosina. Todos los datos se analizaron mediante una prueba t independiente ($p<0,05$), que se implementó con base en la prueba de Kolmogorov-Smirnov y Levene ($p>0,05$).

Resultados: Las mayores expresiones de bFGF y números de osteoblastos se encontraron en el Grupo P14. Hubo diferencias significativas en la expresión de bFGF y el número de osteoblastos en el lado de tensión del hueso alveolar durante la OTM entre el grupo de control y los grupos de tratamiento ($p<0.05$).

Conclusiones: La suplementación posterior de *B. bifidum* muestra la mejora de la expresión de bFGF y el número de osteoblastos en el lado de tensión del hueso alveolar durante la OTM determinada inmunohistoquímicamente.

Palabras Clave: *Bifidobacterium bifidum*; factor de crecimiento de fibroblastos básico; movimiento dental de ortodoncia; osteoblasto.

ARTICLE INFO
Received: January 3, 2021.
Received in revised form: February 16, 2021.
Accepted: February 17, 2021.
Available Online: February 18, 2021.

INTRODUCTION

The orthodontic tooth movement (OTM) aims to obtain an ideal tooth alignment and achieve the aesthetic and occlusion functions (Rossini et al., 2015). Teeth can move in the alveolar bone with the application of an orthodontic force due to the mechanical changes in the biological system that cause tension, thereby stimulating the cellular responses in the periodontal ligament and alveolar bone (Nakano et al., 2014).

Any tissue biological reactions might occur during an orthodontic treatment as the result of changes in the applied force distribution in the periodontal tissue. The ideal OTM only can be arranged if the remodeling of alveolar bone around the tooth occurs (Krishnan and Davidovitch, 2006). Meanwhile, alveolar bone remodeling consists of alveolar bone resorption in the compression site by osteoclast and then followed by the alveolar bone formation by osteoblast in the tension side. Significantly, the processes of alveolar bone remodeling, which involves the interaction between osteoblast and osteoclast during OTM is the one that should be considered by the orthodontist (Huang et al., 2014).

The rate of alveolar bone remodeling during OTM in adults is slower than in adolescents due to the growth and development period (Abbing et al., 2020). The orthodontic treatment plan must be considered a local and general condition from a patient's health, especially in periodontal tissue (Cerroni et al., 2018). Nowadays, many researchers develop methods to accelerate OTM to provide shorter treatment duration by employing either surgical or non-surgical approaches. Specifically, the OTM treatment duration rates can be accelerated through the non-surgical approach, such as supplementing an herbal compound and low-laser intensity therapy that can increase osteoclast-osteoblast interaction by the enhancement of expressed biomolecular markers (Nugraha et al., 2020; Narmada et al., 2019; 2020).

Moreover, the immune system regulates the inflammation process, which is very important for OTM. Any applied OTM force may induce the inflammatory response around the tooth, especially periodontal ligament and alveolar bone (Krishnan and Davidovitch, 2006). The excessive or prolonged inflammation during OTM can interrupt the OTM rate due to the increased production of pro-inflammatory cytokines and the decreased production of anti-inflammatory cytokines or growth factors, which may disrupt alveolar bone remodeling (Huang et al., 2014). In addition, any excessive pro-inflammatory cytokine such as TNF-α can induce the resorption of bone and tooth root (Qi et al., 2019).

Fundamentally, previous research found that *Bifidobacterium bifidum* (Tissier 1900) Orla-Jensen 1924 (family *Bifidobacteriaceae*) is considered as one of beneficial probiotic, which can act as a regulator of immunity in the digestive tract through the modification of reactions related to allergies and inflammation (Khailova et al., 2010). *B. bifidum* is gram-positive bacteria that can activate the toll-like receptor-2 (TLR-2), which is an important receptor on the surface of immune cells that recognize the lipoprotein component of gram-positive bacteria (Llewellyn and Foey, 2017). Moreover, *B. bifidum* also can act as immunomodulators of immune response by reducing the excessive production of pro-inflammatory cytokines and increasing the production of anti-inflammatory cytokines through nuclear factor *kappa* B (NF-κB) signaling (Shirasawa et al., 2010). The higher expression of anti-inflammatory cytokines may inhibit the expression of pro-inflammatory cytokines, resulting in the decrease of prolonged inflammation and may induce the growth factor that is essential for the tissue regeneration process. Consequently, the enhancement of growth factor such as basic fibroblast growth factor (bFGF) is able to stimulate osteoblasts proliferation, maturation, an activity that is important for alveolar bone formation in the tension side during OTM (Inayati et al., 2020). Thus, the purpose of this study is to investigate the bFGF expression and the number of osteoblasts in the tension side of alveolar bone during OTM post *B. bifidum* probiotic supplementation in male Wistar rats.

MATERIAL AND METHODS

Study design and ethical aspects

This study was a true experimental study with a post-test only control group design. The simple blind random sampling method was applied to select the sample in this study. The Health Research Ethics Committee, Faculty of Dental Medicine, Universitas Airlangga Surabaya, East Java, Indonesia, approved this study protocol with the reference number 698/HRECC.FODM/X/2019.

Animal preparation

All experimental procedures involving animals were carried out in accordance with the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines to ameliorate any sufferance of the animals. All the rats were placed individually in polycarbonate cages (0.90 × 0.60 × 0.60 m) for a week on a 12-h light/dark cycle at a steady temperature of 25°C and 50% humidity for the acclimatization to compensate their various origins. Animals were fed with a standard pellet diet with *ad libitum* tap water and routinely inspected for food consumption and fecal characteristics.

Bifidobacterium bifidum probiotic preparation

B. bifidum was obtained from Food and Nutrition Center in Gadjah Mada University, Yogyakarta Special Region, Indonesia. *B. bifidum* BRL 130 strain was confirmed with certificate number of PSPG/0309/IV/19. The animals of the probiotic group were then orally administrated with 10 mL/day of *B. bifidum* in drinking water at the dose of 1.5 x 10^8 CFU/mL every day.

Orthodontic tooth movement animal model and *Bifidobacterium bifidum* probiotic supplementation

The samples of this study consisted of 42 healthy male Wistar rats, around 16-20 weeks-old, with body weight around 200-250 g. The Wistar rats were used as an OTM animal model. All samples were then assigned into six groups (n = 7), respectively: Group 1: OTM and phosphate buffer saline (PBS) (OneMed, Sidoarjo, Indonesia) for 3 days (K3); Group 2: OTM and PBS for 7 days (K7); Group 3: OTM and PBS for 14 (K14); Group 4: OTM and *B. bifidum* for 3 days (P3); Group 5: OTM and *B. bifidum* for 7 days (P7); Group 6: OTM and *B. bifidum* for 14 days (P14). OTM in the animal model was established by using an OTM device consisted of a 6 mm long nickel-titanium open coil spring (American Orthodontics Corp., Sheboygan, WI, USA) placed between the maxillary incisors and the maxillary molar to move the molar mesially with 10 g force and measured by employing the tension gauge (American Orthodontics Corp., Sheboygan, WI, USA). Furthermore, the fixed orthodontic appliance was fixated by applying 0.07 stainless steel ligature wire (American Orthodontics Corp., Sheboygan, WI, USA) that is shown in Fig. 1.

Figure 1. Orthodontic tooth movement (OTM) in the animal model.

The supplementation of *B. bifidum* was completed per orally by utilizing the oral gavage, consisting in a stomach tube with a single dose of 1.5 × 10^8 CFU/mL every day based on the previous study (Pazzini et al., 2017). Animals were then sacrificed after days 3, 7, and 14 by a lethal dose of rodent anesthesia (60 mg/body weight of ketamine and 3 mg/body weight of xylazine [Sigma Aldrich, Merck KGaA, Darmstadt, Germany]). The rats' maxillae were then dissected and placed in 10% formalin (OneMed, Surabaya, Indonesia) for four days, and then they were decalcified for 3 months by applying ethylenediaminetetraacetic (EDTA) (OneMed, Surabaya, Indonesia). Thus, the sample underwent tissue processing for further immunohistochemical analysis (IHC) analysis.

Moreover, IHC staining using a 3,3-diaminobenzidine stain kit (DAB) (Sigma Aldrich, Merck KGaA, Darmstadt, Germany) was performed. Anti-bFGF (SantaCruz Biotechnology Inc., Dallas, TX, USA) antibody monoclonal (AbMo) was also utilized. Hematoxylin and eosin staining (Sigma Aldrich, Merck KGaA, Darmstadt, Germany) was applied as staining for the osteoblast examination. The observation and examination of expression from bFGF and the number of osteoblasts in the tension side of alveolar bone were carried out by two observers in the different visual fields by applying Nikon 600L inverted light microscope (Nikon Corp., Tokyo, Japan) at 1000× magnification (Nikon Corp., Tokyo, Japan). Then all data were recapitulated for further analysis.

Statistical analysis

All data were analyzed by using Statistical Package for the Social Sciences 20.0 software (SPSS for Windows, Chicago, USA). Descriptive statistics were described as mean ± standard deviation (SD). An independent t-test was implemented between K3 group and T3 group, K7 group and T7 group, K14 group and T14 group ($p<0.05$) based on Kolmogorov-Smirnov and Levene's test ($p>0.05$).

RESULTS

All data were homogenous and normally distributed ($p>0.05$). The result of IHC examination of bFGF expression can be seen in Fig. 2A-F; meanwhile, treatment groups (D-F) showed more positive expression (brown colored) compared to control groups (A-C). The expression of bFGF increased in the treatment groups and the highest was found in the P14 treatment group (Fig. 3). The result of osteoblast number can be seen in Fig. 4A-F; in which the treatment groups (D-F) were compared to the control groups (A-C). The number of osteoblasts increased in the treatment group and the highest one was found in the P14 treatment group (Fig. 5). There were significant differences in bFGF expression and osteoblast numbers in the tension side of alveolar bone during OTM between the control groups and the treatment groups respectively: K3 and P3; K7 and P7; K14 and P14 ($p<0.05$).

DISCUSSION

In this study, we found that the supplementation of *B. bifidum* as a beneficial probiotic can increase the bFGF expression and the number of osteoblasts in the tension side of alveolar bone during OTM in Wistar rats from days 3, 7 and 14, immunohistochemically. The highest expression of bFGF and number of osteoblasts were found in the T14 group. The bFGF expressions and osteoblast number between OTM and PBS only group or OTM and *B. bifidum* supplementation group were significantly different. The continuous application of orthodontic mechanical forces induces the inflammatory processes through toll like receptor-4 (TLR4)/NF-κB signaling, which induces stress on the vascularization in the periodontal ligament. Any excessive pressure results in ischemia, gradually decreasing capillaries, thrombi, nutritional disturbances, cell death and necrotic or hyalinization in the pressure side. Conversely, dilation of blood vessels occurs in the tension side. Furthermore, changes in vascularity are mediated by growth factors, such as bFGF and vascular endothelial growth factor (VEGF) (Krishnan and Davidovitch, 2006). Moreover, bFGF increases the proliferation of endothelial cells and causes endothelial cell growth. bFGF is also a component of the bone matrix, playing an important role in regulating bone remodeling. Any increase in bFGF

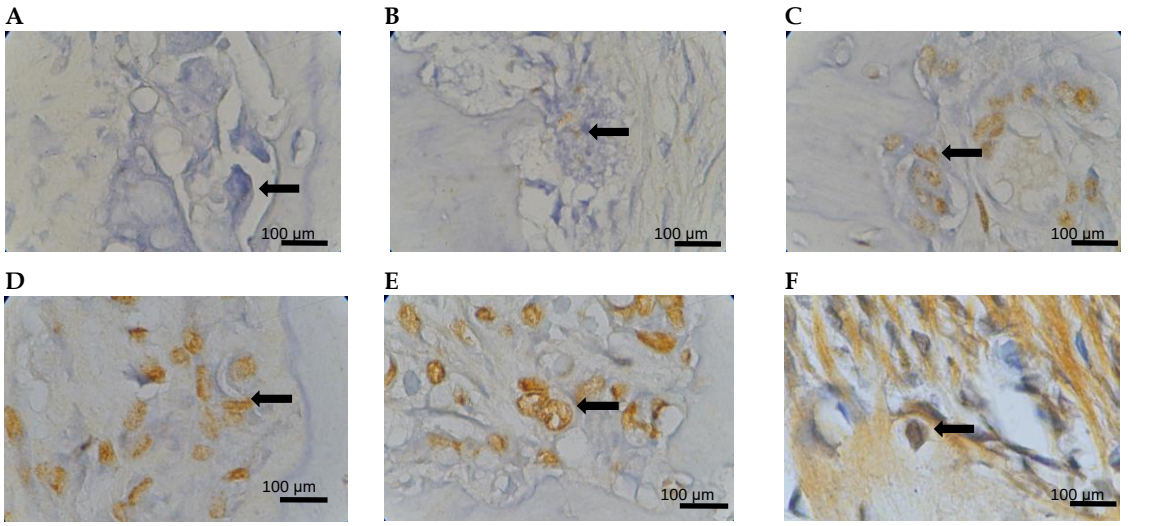

Figure 2. Positive expressions of bFGF in the osteoblast in the tension side of alveolar bone during OTM in male Wistar rats.

bFGF expression in: **(A)** tension side of OTM and PBS for 3 days (K3) group; **(B)** tension side of OTM and PBS for 7 days (K7) group; **(C)** tension side of OTM and PBS for 14 (K14); **(D)** in tension side of OTM and *B. bifidum* for 3 days (P3) group; **(E)** tension side of OTM and *B. bifidum* for 7 days (P7); **(F)** tension side of OTM and *B. bifidum* for 14 days (P14). Black arrows in all groups indicate positive expression of bFGF. Magnification 1000×.

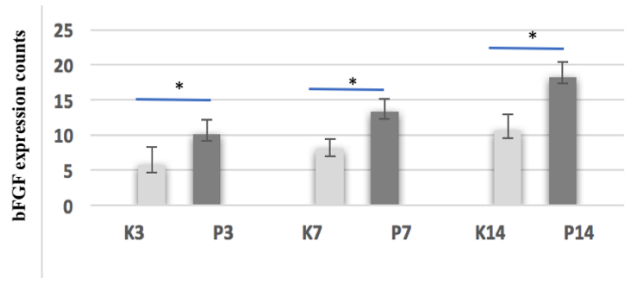

Figure 3. The number of basic fibroblast growth factor (bFGF) expressions in osteoblast of alveolar bone in the tension side during OTM in male Wistar rats.

Data are expressed as mean ± SD (n = 7), *p<0.05 statistically significant differences between control groups and treatment groups. K3: The groups were OTM and PBS for 3 days; K7: OTM and PBS for 7 days; K14: OTM and PBS for 14; P3: OTM and *B. bifidum* for 3 days; P7: OTM and *B. bifidum* for 7 days; P14: OTM and *B. bifidum* for 14 days.

expression in the tension side of alveolar bone during OTM might be correlated with periodontal tissue remodeling (Inayati et al., 2020). The process of remodeling both in the periodontal ligament and alveolar bone involves an inflammatory reaction, which occurs as a typical body reaction to counteract the destructive stimulus and regenerate the injured tissues. Thus, bFGF is a potent angiogenic factor as its expression increases under hypoxic conditions and during the tissue regeneration (Li et al., 2018).

The significant increase in bFGF expression in the treatment groups of this study is in accordance with the opinion that many signaling molecules in the TLR4 or TLR2/NF-κB pathway provide an opportunity for probiotics to modulate the inflammatory responses, including alveolar bone remodeling during OTM. Furthermore, TLR2 activation by the probiotics can induce a suppressive effect on TLR4-mediated inflammatory response (Hachimura et al., 2018). The inhibition of NF-κB signaling may provide a feedback mechanism against the active Wnt signaling, which activates runt-related transcription factor 2 (RUNX2) and Osterix that increases the osteogenic differentiation of mesenchymal stem cell into osteoblast

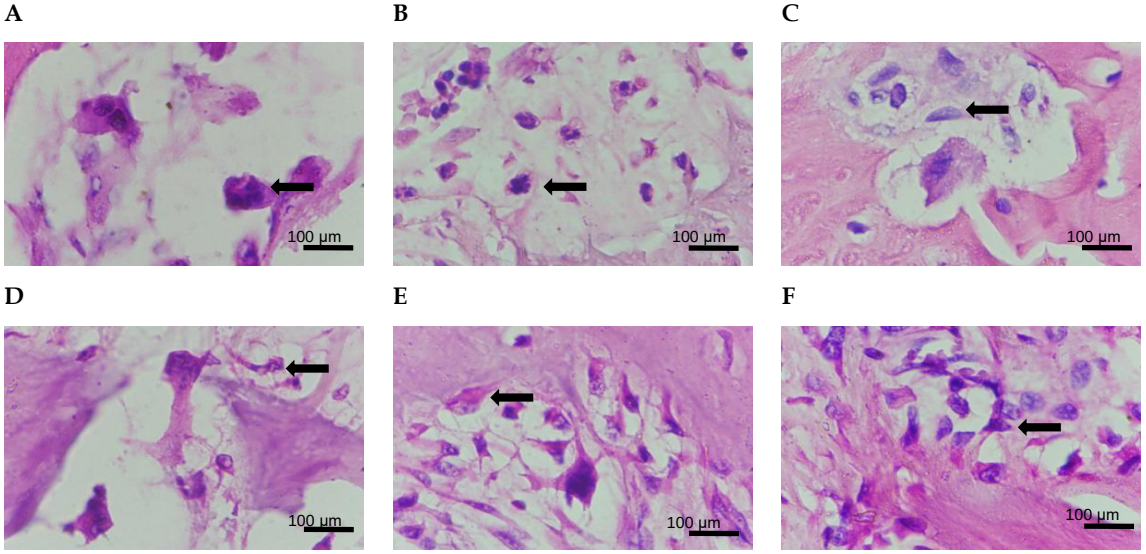

Figure 4. The osteoblast cells (black arrow) were found in the tension side of alveolar bone during OTM.
(A) OTM and PBS for 3 days (K3); **(B)** OTM and PBS for 7 days (K7); **(C)** OTM and PBS for 14 (K14); **(D)** OTM and *B. bifidum* for 3 days (P3); **(E)** OTM and *B. bifidum* for 7 days (P7); **(F)** OTM and *B. bifidum* for 14 days (P14). Hematoxylin and eosin staining, magnifications 1000×.

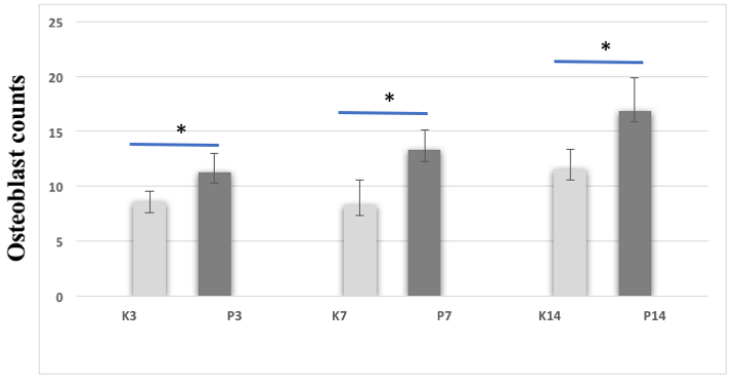

Figure 5. The number of osteoblasts in the tension side of alveolar bone during OTM in male Wistar rats.
Data are expressed as mean ± SD (n = 7), *p<0.05 statistically significant differences between control groups and treatment groups. K3: OTM and PBS for 3 days; K7: OTM and PBS for 7 days; K14: OTM and PBS for 14; P3: OTM and *B. bifidum* for 3 days; P7: OTM and *B. bifidum* for 7 days; P14: OTM and *B. bifidum* for 14 days.

(Sitasari et al., 2020). The osteoclast number decreases but the osteoblast number increases during OTM post oral supplementation of probiotic (Pazzini et al., 2017). The decreased inflammatory cytokine and the activation of NF-κB inhibit the expression of nuclear factor-associated T cells-1 (NFATc1) and sclerostin during OTM (Hernawan et al., 2020). The data analysis shows that bFGF expression in the treatment group on day 14 rises more significantly than in the control group. This result showed that the systemic *B. bifidum* supplementation significantly affects the increasing bFGF expression in the tension side. The alveolar bone formation during OTM is about 6 to 15 days (Krishnan and Davidovitch, 2006). Thus, in this time frame, there are many growth factors expressed in osteoblast or fibroblast in the periodontal tissue (Li et al., 2018).

The significant increase in bFGF expression on day 14 in the treatment group expresses that the *B. bifidum* probiotic exhibits its role to enhance the alveolar bone formation in the tension side during OTM. The local application of probiotics as much as 50 µg/mL can enhance the regeneration of wounds in animal models. The signaling pathway of PI3K/AKT/β-catenin/transforming growth factor β1 is activated after the local application of

probiotics. Then, it can increase the wound regeneration rate *in vivo* (Han et al., 2019). This study's limitation is covered in OTM animal model with limited sample size, time frame, and examination method only. Further study is still required to elucidate the mechanism of alveolar bone remodeling during OTM after *B. bifidum* supplementation.

CONCLUSIONS

Post supplementation *of B. bifidum* shows the enhancement of bFGF expression and osteoblast number in the tension side of alveolar bone during OTM, immunohistochemically.

CONFLICT OF INTEREST

The authors declare no conflicts of interests.

ACKNOWLEDGMENTS

This study was funded by Penelitian Unggulan Fakultas supported by Airlangga University. The authors express the sincerest gratitude to the Faculty of Dental Medicine authorities, Surabaya, Indonesia, with appointment number: 346/UN3/2020.

REFERENCES

Abbing A, Koretsi V, Eliades T, Papageorgiou SN (2020) Duration of orthodontic treatment with fixed appliances in adolescents and adults: A systematic review with meta-analysis. Prog Orthod 21(1): 37.

Cerroni S, Pasquantonio G, Condò R, Cerroni L (2018) Orthodontic fixed appliance and periodontal status: An updated systematic review. Open Dent J 12: 614–622.

Hachimura S, Totsuka M, Hosono A (2018) Immunomodulation by food: Impact on gut immunity and immune cell function. Biosci Biotechnol Biochem 82(4): 584–599.

Han N, Jia L, Su Y, Du J, Guo L, Luo Z, Liu Y (2019) *Lactobacillus reuteri* extracts promoted wound healing via PI3K/AKT/β-catenin/TGFβ1 pathway. Stem Cell Res Ther 10(1): 243.

Hermawan RW, Narmada IB, Djaharu'ddin I, Nugraha AP, Rahmawati D (2020) The Influence of epigallocatechin gallate on the nuclear factor associated T cell-1 and sclerostin expression in Wistar rats (*Rattus novergicus*) during the orthodontic tooth movement. Res J Pharm Technol 13(4): 1730–1734.

Huang H, Williams RC, Kyrkanides S (2014) Accelerated orthodontic tooth movement: molecular mechanisms. Am J Orthod Dentofacial Orthop 146(5): 620–632.

Inayati F, Narmada IB, Ardani IGAW, Nugraha AP, Rahmawati D (2020) Post oral administration of epigallocatechin gallate from *Camelia sinensis* extract enhances vascular endothelial growth factor and fibroblast growth factor expression during orthodontic tooth movement in Wistar rats. J Krishna Inst Medical Sci Univ 9(1): 58–65.

Khailova L, Mount Patrick SK, Arganbright KM, Halpern MD, Kinouchi T, Dvorak B (2010) *Bifidobacterium bifidum* reduces apoptosis in the intestinal epithelium in necrotizing enterocolitis. Am J Physiol Gastrointest Liver Physiol 299(5): G1118–G1127.

Krishnan V, Davidovitch Z (2006) Cellular, molecular, and tissue-level reactions to orthodontic force. Am J Orthod Dentofacial Orthop 129(4): 469.e1-32.

Llewellyn A, Foey A (2017) Probiotic modulation of innate cell pathogen sensing and signaling events. Nutrients 9(10): 1156.

Li Y, Jacox LA, Little SH, Ko CC (2018) Orthodontic tooth movement: The biology and clinical implications. Kaohsiung J Med Sci 34(4): 207–214.

Nakano T, Hotokezaka H, Hashimoto M, Sirisoontorn I, Arita K, Kurohama T, Darendeliler MA, Yoshida N (2014) Effects of different types of tooth movement and force magnitudes on the amount of tooth movement and root resorption in rats. Angle Orthod 84(6): 1079–1085.

Narmada IB, Rubianto M, Putra ST (2019) The role of low-intensity biostimulation laser therapy in transforming growth factor β1, bone alkaline phosphatase and osteocalcin expression during orthodontic tooth movement in *Cavia porcellus*. Eur J Dent 13(1): 102–107.

Narmada IB, Rubianto M, Putra ST (2020) The effect of low-level light therapy on orthodontic tooth movement rate, heat shock protein 70, and matrix metallopreteinase 8 expression: Animal study. Dent Res J (Isfahan) 17(1): 73–79.

Nugraha AP, Narmada IB, Sitasari PI, Inayati F, Wira R, Triwardhani A, Hamid T, I Gusti Aju Wahju Ardani IGAW, Djaharu'ddin I, Rahmawati D, Iskandar RPD (2020) High mobility group box 1 and heat shock protein-70 expression post (-)-epigallocatechin-3-gallate in East Java green tea methanolic extract administration during orthodontic tooth movement in Wistar rats. Pesqui Bras Odontopediatria Clín Integr 20: e5347.

Pazzini CA, Pereira LJ, da Silva TA, Montalvany-Antonucci CC, Macari S, Marques LS, de Paiva SM (2017) Probiotic consumption decreases the number of osteoclasts during orthodontic movement in mice. Arch Oral Biol 79: 30–34.

Qi J, Kitaura H, Shen WR, Kishikawa A, Ogawa S, Ohori F, Noguchi T, Marahleh A, Nara Y, Mizoguchi I (2019) Establishment of an orthodontic retention mouse model and the effect of anti-c-Fms antibody on orthodontic relapse. PLoS One 14(6): e0214260.

Rossini G, Parrini S, Castroflorio T, Deregibus A, Debernardi CL (2015) Efficacy of clear aligners in controlling orthodontic tooth movement: A systematic review. Angle Orthod 85(5): 881–889.

Shirasawa Y, Shibahara-Sone H, Iino T, Ishikawa F (2010) *Bifidobacterium bifidum* BF-1 suppresses *Helicobacter pylori*-

induced genes in human epithelial cells. J Dairy Sci 93(10): 4526–4534.

Sitasari PI, Narmada IB, Hamid T, Triwardhani A, Nugraha AP, Rahmawati D (2020) East Java green tea methanolic extract can enhance RUNX2 and Osterix expression during orthodontic tooth movement *in vivo*. J Pharm Pharmacogn Res 8(4): 290–298.

AUTHOR CONTRIBUTION:

Contribution	Triwardhani A	Anggitia C	Ardani IGAW	Nugraha AP	Riawan W
Concepts or ideas	x				
Design	x				
Definition of intellectual content		x			
Literature search		x			
Experimental studies		x			
Data acquisition		x			
Data analysis	x	x		x	x
Statistical analysis	x	x		x	
Manuscript preparation	x	x	x	x	x
Manuscript editing	x	x	x	x	x
Manuscript review	x	x	x	x	x

Citation Format: Triwardhani A, Anggitia C, Ardani IGAW, Nugraha AP, Riawan W (2021) The increased basic fibroblast growth factor expression and osteoblasts number post *Bifidobacterium bifidum* probiotic supplementation during orthodontic tooth movement in Wistar rats. J Pharm Pharmacogn Res 9(4): 446–453.