The Number of Osteoblast and Osteoclast during Orthodontic Tooth Movement after Preconditioned Gingiva Mesenchymal Stem Cell Allogeneic Transplantation *in vivo*

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Abstract

Malocclusion can affect the quality of life related to oral health. A novel technique to expedite orthodontic tooth movement (OTM) in order to minimize treatment length and the side effects. Under orthodontic fore, MSCs transplantation might hypothetically accelerate the bone remodeling process, resulting in OTM acceleration.

To investigate the number of osteoclasts and osteoblasts in the tension side during OTM after transplantation of normoxic or hypoxic preconditioned allogeneic gingival mesenchymal stem cells in vivo.

The OTM animal model was 48 male rabbits (Oryctolagus cuniculus) aged 6 months with body weight about 3-4 kg. There were 4 experimental groups in this study: control negative group (C-): injected with PBS without OTM, positive control group (C+): 50g OTM with 20µL PBS injection, treatment group 1 (T1): 50g OTM with 20µL GMSCs normoxia in PBS, treatment group 1 (T2): 50g OTM with 20µL GMSCs hypoxia in PBS. The injection was done in afflicted mandibular gingiva using µL small needle syringe with local infiltration technique. Every sample then was sacrificed after day 7, 14, and 28 respectively. The number of osteoblasts and osteoclasts in the alveolar bone during OTM was determined by hematoxylin and eosin staining.

In T2 group, osteoblast was significantly enhanced on day 14 and 28 but not osteoclast number during OTM. There was significant different in osteoclast and osteoblast number between groups ($p<0.05$)

GMSCs hypoxia preconditioned escalate osteoblast number but not decrease the osteoclast number in the tension side during orthodontic tooth movement in vivo.

Experimental article (J Int Dent Med Res 2022; 15(3): 1069-1077)

Keywords: Dentistry, tooth movement technique, orthodontics appliance, medicine, bone remodeling.

Received date: 01 June 2022 **Accept date:** 04 July 2022

Introduction

Malocclusion, or crowded, irregular, and prominent teeth, has been an issue for many people since ancient times, and efforts to treat it have been attempted since at least 1000 BC. Malocclusion is a condition in which the teeth of the upper and lower jaws are positioned improperly. Malocclusion is also an indication of a discordant connection between the jaw and teeth as a result of their shape or size.[1]

Angle's categorization has been utilized by the worldwide community to examine the mesio-distal connections of the dental arches since 1907. It serves as the foundation for determining whether or not a malocclusion in the

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sagittal dimension requires orthodontic treatment. The examination of the occlusal anomaly of setting (centering or guiding) and the category of occlusion (functional occlusion, functional malocclusion, or pathogenic malocclusion), ie its probable pathogenic influence on orofacial structures, must complete the kind of abnormalities (skeletal and dental).[2] Malocclusions are now considered the third most common oral health condition by the World Health Organization, behind dental caries and periodontal diseases.[3]

The frequency of dental and oral health is still very high in Indonesia. Dental and oral diseases impact 57.6 percent of the population, according to the findings of the 2018 National Basic Health Research. Malocclusion is one of the remaining dental and oral disorders in Indonesian population.[4] Malocclusion is one of the dental issues that ranks third in terms of dental and oral health concerns in Indonesia, following dental caries and periodontal disease, with an estimated incidence of around 80%. The high percentage is due to a lack of understanding about dental care.[5]

Malocclusion can affect the quality of life related to oral health (oral health related to quality of life / OHR-QOL). malocclusion is accompanied by a significant increase in overjet related to complaints in the oral cavity, functional limitations of Stomatognathic, and social welfare. Inner bite is also significantly related to oral complaints and functional limitations of Stomatognathic.[6] The socioeconomic situations and aesthetic implications of malocclusion have a significant impact on the quality of life of teenagers.[7]

Orthodontic tooth movement (OTM) is defined as a biological response to a change in the physiological balance of the dentofacial complex induced by an applied external force to treat malocclusion. OTM occurs in the force direction, with bone resorption in the compression area and bone apposition in the tensile area of the periodontal ligament.[8] OTM is induced by tissue remodeling around the tooth root as a result of force application. Remodeling requires the existence of cells capable of absorbing and producing the extracellular matrix of the periodontal ligament and alveolar bone. When orthodontic stresses are applied, the stressed side's periodontal ligament constriction is followed by alveolar bone loss owing to osteoclast activity. On the other hand, osteoblasts appoint bone on the tensile side.[9]

As modern dentistry evolves, the necessity for quicker orthodontic care and the investigation of methods to deliver the finest form of patient care becomes increasingly desirable. A multitude of minimally invasive surgical and non-surgical treatment options have been developed as a result of extensive study to help speed tooth motions. Although this method shows promise in terms of treatment acceleration, it does not have the ability to improve periodontal outcomes.[10] Surgical treatment time for accelerating the movement of orthodontic treatment depends on the speed of the movement of the tooth which in turn depends on the speed of alveolar bone remodeling. Therefore, it is suspected that an increase in the speed of teeth movement can be achieved by accelerating the biological response of periodontal ligaments and alveolar bones.[11]

Comprehensive orthodontic therapy is time-consuming, but the best data at this time, based on prospective studies done in a university research setting, indicates that it takes less than two years on average to complete.[12] As treatment advances, the risk of root resorption and decalcification increases. However, because the patient desires a much shorter treatment period of only 6-12 months, Orthodontist, the Orthodontic company's material, has performed considerable research to create ways to accelerate therapy. Some orthodontic firms are currently marketing products, materials, and processes that claim to reduce treatment durations in half.[13]

Corticosteroids, vitamin D3, parathyroid hormones, and thyroxine have all been demonstrated in animal studies to stimulate tooth mobility. Human investigations examining the use of prostaglandins in the movement of orthodontic teeth discovered the possibility of increasing teeth movement. As a result, medicines that inhibit the movement of orthodontic teeth, such as aspirin and NSAIDs, have been demonstrated in animal studies to slow the movement of orthodontic teeth.[14] Another method that has been promoted is the use of autologous plasma rich platelets (PRP) as a substitute for cytokines or local medications to recreate the effects induced in the bone following surgery. Platelets, which include growth factors such as Platelet Derived Growth Factor (PDGF) and Transforming Growth Factor (TGF), are the starting point for

soft and hard tissue wound healing (TGF).[13]

Mesenchymal stem cells (MSCs) are self-renewing cells that can develop into a variety of cells under the right conditions.[15] MSCs have been found in muscles, dermis, bone marrow, adipose tissue, periosteum, blood, umbilical cord, synovial membrane, and teeth.[16,17] Some of these sources are easily accessible in orthodontic since excision of oldest teeth, permanent premolar teeth, or wisdom teeth is a common intervention in the treatment of malocclusion orthodontic, MSCs sources from teeth may be collected without further morbidity. Several investigations have indicated the ability of mesenchymal stem cells (MSCs) derived from dental pulp, periodontal ligaments, or exfoliated deciduous tooth to differentiate and proliferate.[18-22]

Defects treated with bone marrow-derived mesenchymal stem cells (BM-MSCs) regenerate more faster and had better reepithelization, cellularity, and angiogenesis.[23]

Orthodontists and researchers have been seeking for novel techniques to expedite OTM in order to minimize treatment length, side effects such as pain, discomfort, dental caries, and periodontal disease, as well as iatrogenic damage such as root resorption, teeth becoming non-vital, and adverse effects. [24] During the application of orthodontic force, an increase in periodontal ligament (PDL) progenitor cells was observed by pressing the expression of type I (Col-I) collagen expression, while after the forced withdrawal with orthodontic appliance increasing the Col-I expression, indicating that periodontal ligament stem cells (PDLSC) is able to respond to the orthodontic mechanical strength with collagen expressions pressed.[25] The ability of this MSCs can be employed to accelerate OTM in response to orthodontic force. When orthodontic force is applied, tooth movement is halted until the necrosis is eliminated, resulting in clinical symptoms of the lag phase. Under pressure, MSCs transplantation might hypothetically accelerate the process, resulting in OTM acceleration.[26]

Bone remodeling is the removal of old or damaged bones by osteoclasts and the formation of new bones by osteoblasts. Osteoclasts are bone absorbent cells that disintegrate bone via acid secretion and proteolytic enzymes such as Cathepsin K (CTSK), which dissolves collagen and other matrix proteins during bone resorption.[27-29] Meanwhile, osteoblasts are bone-forming cells that develop from mesenchymal precursors with osteoprogenitor derivatives via transcription factor activation and eventually differentiate into osteocytes.[30,31]

Extracellular proteins produced by osteoblasts include osteocalcin, alkaline phosphatase, and type I collagen, which accounts for more than 90% of bone matrix proteins. Extracellular matrix is first produced as an osteoid, which is then terminalized by the buildup of calcium phosphate in the form of hydroxyapatite.[32] To control cell proliferation, differentiation, and survival, osteoblasts and osteoclasts have direct contact via the interaction of EFNB2-EPHB4, FAS-FASL, and NRP1-SEMA3A. TGF-b and IGF-1 are released from the bone matrix by osteoclasts, allowing osteoblasts to initiate bone growth. Despite the fact that numerous variables have been found that influence the interaction of osteoblasts and osteoclasts, relatively little from this route has resulted in therapy authorized for the treatment of bone metabolism.[33] Furthermore, the number of osteoclasts and osteoblasts in the tensile side during OTM following transplantation of normoxic or hypoxic preconditioned allogeneic gingival mesenchymal stem cells in young male rabbit (Oryctolagus cunniculus) was investigated.

Materials and methods

Ethical Clearance Statement

All techniques in this study were carried out in compliance with the applicable standards and regulations established by the Ethics Committee of the Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.

Experimental Study Design

This study was true experimental laboratory study with post-test only control group design. The sample was selected blind randomly and the sample size was determined using minimal sample size formula. A sample of 48 male rabbits (*Oryctolagus cuniculus*) aged 6 months with body weight ranging from 3-4 kg and declared healthy by physical examination and having a complete dentition and healthy periodontium were carried out by veterinarian. Each group consists of 4 samples. Each rabbit was kept in a cage measuring 60 x 40 x 40 cm and adapted for 1 week before treatment. Each sample was kept in a controlled temperature

room (22 ± 2°C) with a 12-hour dark and light cycle and was able to access feed and drink according to standards. There were 4 experimental groups in this study: control negative group (C-): injected with PBS without OTM, positive control group (C+): OTM with 20µL PBS injection, treatment group 1 (T1): OTM with 20µL GMSCs normoxia in PBS, treatment group 1 (T2): OTM with 20µL GMSCs hypoxia in PBS. The injection was done in afflicted mandibular gingiva using µL small needle syringe (Terumo, US) with local infiltration technique.

Application of Orthodontic Tooth Movement

OTM appliances was installed between the incisor and mandibular molars using a 6 mm NiTi close coil spring (American Orthodontics Corp., Sheboygan, WI, USA) to move the molar mesially with 50g mild force of OTM and measured with a tension gauge (American Orthodontics Corp., Sheboygan, WI, USA). 0.07 stainless steel ligature wire (American Orthodontics Corp., Sheboygan, WI, USA) was used to secure the fixed orthodontic device. To decrease discomfort in the animals during treatment, an anesthetic was provided intravenously (3 percent pentobarbital sodium, 1 ml/kg).[34] Every sample then was sacrificed through slaughter by means of sharp blade after day 7, 14, and 28 respectively.

Gingiva Mesenchymal Stem Cell culture, sub-culture, confirmation, hypoxia preconditioned and transplantation

The GMSC isolation process was carried out in accordance with method conducted by Nugraha et al.[35] GMSCs were extracted from the gingiva of a healthy male rabbit (*O. cuniculus*) aged 6 months and weighing 3-5 kg in the lower jaw area using a scalpel and blade. The gingiva was chopped into tiny pieces and grown in Dulbeccos Modified Eagle Medium (DMEM, Life Technologies/GIBCO BRL) with 20% Fetal Bovine Serum (FBS, Biochrom AG, Germany), 5m M L - glutamine (Gibco Invitrogen), 100 U/ml penicillin-G, 100 g/ml streptomycin, and 100 g/ml kanamycin. After three days, the medium was withdrawn in order to eliminate the cell components that were not connected to the dish, and a fresh media was provided. FGF-2 was introduced at this point. After the cells were confluent, they were passaged with 0.05 percent trypsin-EDTA, washed, and cultivated again in 60- or 100-mm tissue culture dishes (Corning). The confluent cells were re-passed, and the cells were ready for further study. If the cells are not used right away, they should be kept in liquid N2. Thawing GMSCs begins with removing the cell vial from the freezer. The vial was immersed in a 37oC water bath for 1 minute, until the cells started to melt. Make certain that the bottle does not sink and that no water enters the bottle. To sterilize the vial, it is withdrawn from the water bath and wiped with 70% isopropanol or ethanol. Cells were transferred into a 15-ml tube containing 10 ml of Iscove's Modified Dublbecco's Media (IMDM) + 2% Fetal Bovine Serum using a sterile 2 ml pipette (FBS).

The cells were centrifuged and discarded, and the cell pallet was resuspended in an initial volume of IMDM +2 percent FNS and the total cell volume to be cultivated up to the 4th passage according to study needs. GMSCs are MSCs isolated from the free gingival margins of experimental animals and have the capacity to differentiate mesenchymal lineages such as osteogenic differentiation proven by the Alizarin's Red staining procedure positive characterized by mineral deposits.[36] Adaptive GMSCs were passage 4 GMSCs that had been hypoxia preconditioned by treating them in vitro for 24 hours with 100 M Cobalt Chromium (CoCl2) as a hypoxia mimicking agent (HMA).[37] After 1 day of PGO, hypoxic GMSCs of 10^6 cells in L in PBS were administered in a single dose with a tiny needle syringe (Terumo, US) using a local infiltration approach into the gingiva of male rabbits (*O. cuniculus*).

Hematoxylin eosin staining to examine osteoblast and osteoclast number

The rabbit's mandibular of each sample was dissected and put in 10% neutral buffer formalin (OneMed, Surabaya, Indonesia) for four days before being decalcified for three months with ethylenediaminetetraacetic acid (EDTA) (Leica, Germany). The number of osteoblasts and osteoclasts in the alveolar bone during OTM was determined by mounting histological slices of samples on glass microscope slides and staining them with hematoxylin and eosin (Sigma Aldrich, St Louis, MO, USA). The number of osteoblasts and osteoclasts from the five images were first accumulated for each rabbit, with the mean of the five sections then calculated under 400x magnifications by means of an Olympus Light Microscope (CX23, Olympus, New York, USA) from five different fields of view.[38,39]

Statistical analysis

The research data was then summarized and analyzed descriptively and inferentially. A bar chart containing the mean and standard deviation is used to display the data. The data were analyzed using the statistical package for social science (SPSS) version 20.0 for Windows, which included the normality and homogeneity test (p> 0.05), the analysis of variance (ANOVA) difference test, and the post-hoc Tukey Honest Significant Different (HSD) with a p0.05 significance value (IBM corporation, Ilinois, Chicago, US).

Results

Osteoblasts and osteoclasts were found on histopathological examination in all groups (C- ; C+, T1, T2). The morphology of osteoblasts is single-nucleated, round cells located on the surface of the alveolar bone, whereas the morphology of osteoclasts is large, multinucleated cells located on the bone surface accompanied by resorption areas of osteoblasts (green arrows) and osteoclasts (red arrows). seen with a light microscope at 400x magnification (Figure 1).

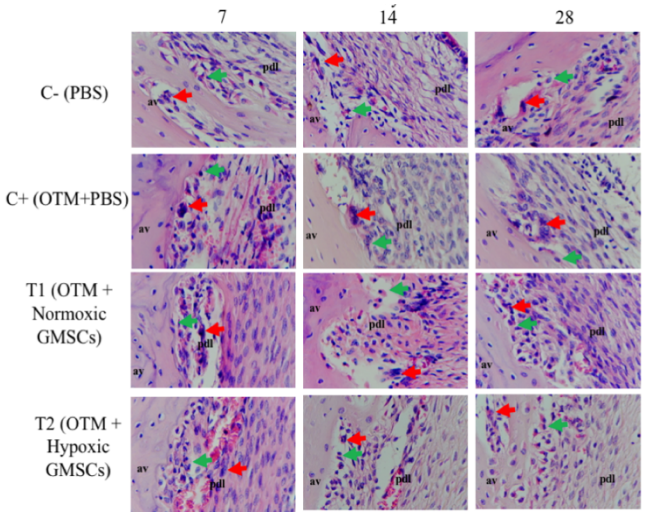

Figure 1. Observations on the morphology and quantity of osteoblasts (green arrows) and osteoclasts (red arrows) in alveolar bone using a light microscope at 400x magnification.

Figure 2 depicts the mean and standard deviation (SD) of the number of osteoblasts in each group. On day 28, the highest number of osteoblasts were detected in the treatment group 2 (T2), whereas the lowest number of osteoblasts were found in the negative control group ©on days 7 and 28. The number of osteoblasts differed significantly across the treatment groups and between the observation days. On day 7, there was a substantial difference in the number of osteoblasts between k- and PII. On day 14, the quantity of osteoblasts differed significantly between C- and T2; C+ and T2.

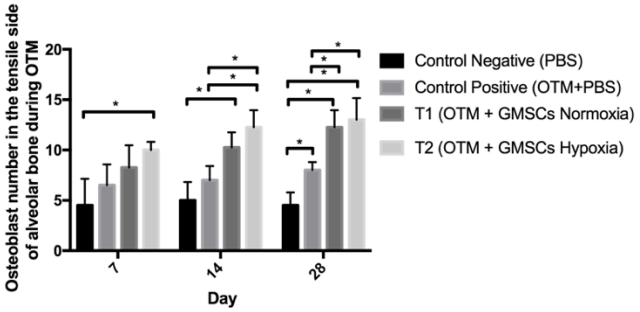

Figure 2. Bar chart of mean and standard deviation (SD) of osteoblast counts between groups. *information: significant at p<0.05 between groups.

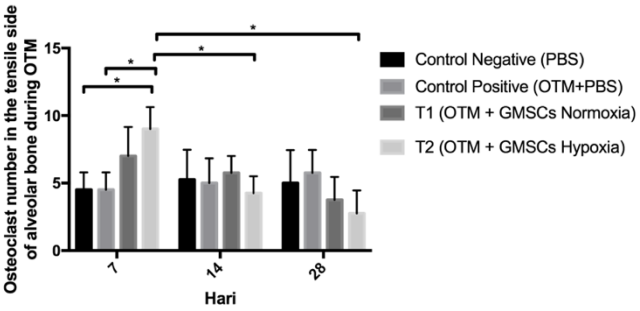

Figure 3. Bar chart of mean and standard deviation (SD) of osteoclast counts between groups. *information: significant at p<0.05 between groups.

Figure 3 shows the mean and standard deviation (SD) of the number of osteoclasts in each group. In this study, the treatment group 2 (T2) had the most osteoclasts on day 7 and the control group (C-) had the fewest on day 28 after treatment 2 (T2). The number of osteoclasts differed significantly between the days of observation. On day 7, there was a substantial difference in the number of osteoclasts between C- and T2, C+ and T2. There was a substantial difference in the number of osteoclasts between T2 on day 7 and T2 on day 14, and T2 on day 14 and T2 on day 28.

Group	Day			p
	7	14	28	
	Mean ± SD			
C-	4.5 ± 2.65[a]	5 ± 1.83[bc]	4.5 ± 1.29[efg]	
Normality test	0.689	0.714	0.972	
C+	6.5 ± 2.08	7 ± 1.41[d]	8 ± 0.82[ehi]	0.0001*
Normality test	0.995	0.161	0.683	
T1	8.25 ± 2.22	10.25 ± 1.5[b]	12.25 ± 1.71[fh]	
Normality test	0.798	0.224	0.850	
T2	10 ± 0.82[a]	12.25 ± 1.71[cd]	13 ± 2.16[gi]	
Normality test	0.683	0.850	0.577	
p-value		0.006*		

Table 1. Description of mean, standard deviation (SD), and test of differences between groups of osteoblast counts.

* information: significant at p<0.05 between groups and days of observation.[abcdefghi] the same superscript showed that there were differences between groups (based on the Tukey HSD test).

Group	Day			p-value
	7	14	28	
	Mean ± SD			
C-	4.5 ± 1.29[a]	5.25 ± 2.22	5 ± 2.45	
Normality test	0.972	0.798	0.262	
C+	4.5 ± 1.29[b]	5 ± 1.82	5.75 ± 1.71	0.852
Normality test	0.972	0.714	0.850	
T1	7 ± 2.16	5.75 ± 1.26	3.75 ± 1.71	
Normality test	0.577	0.406	0.850	
T2	9 ± 1.63[abcd]	4.25 ± 1.26[c]	2.75 ± 1.71[d]	
Normality test	0.683	0.406	0.850	
p-value		0.012*		

Table 2. Description of mean, standard deviation (SD), homogeneity test, normality test and difference test between groups of osteoclast counts.

* information: significant at p<0.05 between days of observation.
[abcdefghi] the same superscript showed that there were differences between groups (based on the Tukey HSD test).

Figure 3 shows the mean and standard deviation (SD) of the number of osteoclasts in each group. In this study, the treatment group 2 (T2) had the most osteoclasts on day 7 and the control group (C-) had the fewest on day 28 after treatment 2 (T2). The number of osteoclasts differed significantly between the days of observation. On day 7, there was a substantial difference in the number of osteoclasts between C- and T2, C+ and T2. There was a substantial difference in the number of osteoclasts between T2 on day 7 and T2 on day 14, and T2 on day 14 and T2 on day 28.

Discussion

The forces created by the OTM that activate the osteoblasts cause alveolar bone apposition on the tension side. MSCs endogen, which are local precursor cells, give rise to osteoblasts. Mature osteoblasts generate osteoid, which is then mineralized in bone. Several enzymes have also been identified as being involved in the production of tensile alveolar bone. Alkaline phosphatase is another another biochemical marker that may be beneficial during osteoblast activity. MSCs can develop into osteogenic differentiation and promote osteoblast proliferation and differentiation on the pull side of the OTM.[24] Previously reported that injection of moringa oleifera extract at 5%, 10%, and 20% concentrations might enhance the number of osteoblasts on the tensile side during OTM in guinea pigs.[40]

Antioxidant activity can boost osteogenic activity, which in turn boosts osteoblastogenesis. Flavonoids can influence cell activity by promoting TGF-b production, whereas TGF-b increases osteoblast proliferation and migration. Increased osteoblast proliferation and activation can increase the number of osteoblasts, which raises the expression of osteoprotegrin (OPG).[41] Increased OPG expression influences osteoclast activation because OPG acts as a receptor for receptor activator of nuclear factor kappa-B ligand (RANKL) by blocking the interaction of RANKL and receptor activator of nuclear factor kappa-B (RANK) on the cell membrane of osteoclast precursors.[42] There are several theories and speculations about the use of stem cells and/or cell therapy as companion therapy during OTM treatment. Despande discovered new bone creation that regenerates like bone in normal settings, with appropriate vascularity and lamellar development, following autologous transplantation of osteoblast culture, which was histopathologically analyzed. Autologous cultivated osteoblast cells had the greatest outcomes in terms of adaptability to various adjuncts, bone regeneration quality, and structural and functional appropriateness.[43]

Progenitor cells inside BMSCs give rise to osteoblasts. BMP, a Wnt signaling pathway ligand, as well as fibroblast growh factor (FGF), insulin growth factor (IGF), and TGF-b, are biological agents that mediate the proliferation and differentiation of osteoblast precursors, eventually encouraging bone production.[44] The activation of key transcription factors, including runt related transcription factor (RUNX2), as well as the activation of the bone morphogenetic protein (BMP) and Wnt signaling pathways, collectively induce osteogenic differentiation of MSCs and initiate osteoblastogenesis. Activated and matured osteoblasts produce osteogenic genes that encode a variety of proteins and

enzymes, including osteonectin, osteocalcin, ALP, and collagen type 1a (Col1a1), all of which are required for the creation of extracellular organic matrix and bone mineralization.[45-48]

MSCs produced from a given tissue maintain some of the'memory' and qualities of the original tissue, exhibiting tissue-specific origin attributes defined by the source microenvironment.[49] MSCs were discovered for the first time in the oral cavity in the periodontal ligament.[50] GMSCs were initially discovered to be heterogeneous and multipotent. During cultivation, human GMSCs were discovered to exhibit high proliferation rates, stable morphology, stable karyotype, and telomerase activit.[51] *In vitro* osteogenic potential of GMSCs has been frequently proven.[52,53]

During OTM, many tissue reactions to bone mechanical stresses occur. The canonical Wnt/-catenin signaling pathway is activated, resulting in transcriptional activation of a variety of osteogenic genes that promote osteoblast differentiation and maturation, as well as bone production. The Wnt/-catenin signaling system may interact with other intracellular signaling pathways induced by prostaglandins, nitric oxide (NO), or BMPs. These interactions aid in the development of bones.[54] The applied force is transmitted through the compressed tissue matrix to local cells in the periodontal ligament and alveolar bone, causing the cells to release pro-inflammatory, angiogenic, and osteogenic agents, triggering the remodeling process of the adjacent alveolar bone and periodontal ligament, allowing tooth movement.[55,56]

The periodontal ligament and alveolar bone can drive enhanced osteogenic gene expression by transforming osteogenic progenitor cells into mature osteoblasts that deposit osteoid and then mineralize it.[55,57] Mechanically produced tensile pressures stimulate endogenous MSCs in alveolar bone tissue, activating the regulated protein kinase ERK 1/2, a member molecule of the mitogen activated protein kinase (MAPK) intracellular signaling cascade. The ERK 1/2 pathway then activates the transcription factor RUNX2, which is a key regulator of osteogenic gene expression throughout the differentiation and maturation of osteogenic precursor cells into osteoblasts that generate ALP, Coll1a1, and osteocalcin.[57,58]

Mechanical tensile pressures can therefore activate the intracellular ERK 1/2-RUNX2 pathway in endogenous MSCs in bone for osteogenic differentiation and facilitate their development into bone-forming osteoblasts.[59] The MAPK intracellular signaling system also comprises the c-JUN N-terminal kinase (JNK) cascade and the p38 cascade, in addition to the ERK 1/2 cascade. The MAPK pathway, namely the p38 cascade, controls the production of inflammatory cytokines and RANKL by osteoblasts, consequently beginning osteoclastogenesis and promoting bone remodeling in periodontal tissues in response to mechanical tensile pressures.[60]

The degree of the tensile strain on the periodontal tissue, which in the context of orthodontic therapy is defined by the properties of the orthodontic force applied, determines the selective activation of intracellular MAPK signaling pathways, whether via ERK 1/2, JNK, or p38.[61] When a tensile force is applied to the tensile side after 72 hours, it looks identical to after 24 hours, but the increase in osteoblasts is accompanied with new bone production.[57] It was discovered in a previous study that moderate hypoxia (10% O_2) appears to boost the rate of proliferation of osteoblast-enriched cells *in vitro.* Hyperoxia (90% O_2), on the other hand, has the opposite effect, as a shift in oxygen tension from low to high leads in a dramatic drop in the rate of osteoblast proliferation. Hypoxia can have a significant impact on bone remodeling.[62] Under these conditions, growth factors and other biologic agents are released from the bone matrix and from squeezed blood vessels, and endogenous MSCs are recruited to bone.[44]

Mechanical stimulation during OTM not only influences bone homeostasis by increasing osteoclastogenesis, but it also influences osteoblastogenesis. Previous research has demonstrated that enhanced osteoblast activity causes upregulation of bone production, which is a frequent impact of OTM on the tensile side.[63]

OTM is primarily mediated by osteoblasts and osteoclasts. Many investigations on osteoclasts and osteoblasts during OTM have been conducted. This pattern of cellular activity that occurs during the differentiation of MSCs into osteoblasts and HSCs into osteoclasts can be used to identify possible indicators linked with OTM. Multinucleated cells called osteoclasts destroy and reabsorb bone. Osteoclasts work with osteoblasts to rebuild bone. Osteoclasts refine their ability to dissolve hydroxyapatite

crystals and destroy organic bone matrix rich in collagen fibers. Osteoclasts are related to immune cells such as megakaryocytes, granulocytes, monocytes, and macrophages. Despite their shared origin, osteoclasts and macrophages in the immune system both have phagocytic roles in bone, thus the term bone-specific macrophages. In pathological situations, osteoclasts act as immunomodulators by secreting different mediators. Osteoclasts have high levels of tartate resistant acid phosphatase (TRAP), Cathepsin K, and OPG expression.[24] A recent research revealed that injection of moringa oleifera extract at 5%, 10%, and 20% doses might lower the number of osteoclasts on the tensile side during OTM in guinea pigs.[40] Antioxidant-active biomaterials can lower the number and activity of osteoclasts.[64] MSCs have antioxidant properties because they regulate the expression of heat shock protein (HSP) and High mobility group box 1 protein (HMGB-1). MSCs have also been linked to the production of regulatory factors that control osteoclast growth and bone resorption.[65] MSCs exhibit immunomodulatory and immunosuppressive properties in vitro and in vivo. In a steroid-induced osteoporosis paradigm, MSC transplantation increases bone matrix synthesis and decreases bone resorption, resulting in an improvement in bone density and structure. MSCs, like other microenvironment-dependent immune cells, have a dual influence on osteoclasts. Preconditioned MSCs implanted in vivo may alter bone osteogenesis potential.[66]

MSC-based regenerative treatment is now popular due to its high potential. Several clinical trials have been attempted to use it to treat bone resorption disease. MSCs inhibited the production of osteoclast development transcription factors by decreasing NF-B and nuclear factor associated T-cell 1 (NFATc1) activity. These findings show that MSC-derived factors limit osteoclast development in RAW264 cells when co-cultured with MSCs, which can also influence osteoclast differentiation via OPG expression. It has been observed that MSCs transplantation was commenced 4 weeks after MSCs transplantation to dramatically accelerate bone regeneration by lowering the number of differentiated osteoclasts. It was discovered that MSCs reduced osteoclast differentiation.[67] This is consistent with the findings of this investigation, which found that preconditioning allogeneic GMSCs transplantation with hypoxia and normoxia can lower the number of osteoclasts on the tension side of the alveolar bone during OTM.

Conclusions

Based on the outcomes of this study, it can be concluded that GMSCs hypoxia preconditioned escalate osteoblast number but decrease the osteoclast number in the tension side during orthodontic tooth movement. However, more research is urgently needed to identify the molecular marker associated with bone resorption and bone apposition during orthodontic tooth movement *in vivo.*

Acknowledgements

The authors would like to thank the Faculty of Medicine at Brawijaya University, as well as the Stem Cell Research Center and Development at Universitas Airlangga, for their assistance and considerate direction in this study. This study was supported by Penelitian Dasar Unggulan Perguruan Tinggi (PDUPT) 2021, with appointment number 275/UN3/2021 from the Ministry of Education, Culture, Research, and Higher Education on March 9, 2021.

Declaration of Interest

The authors declare there is no conflict of interest in this study

References

1. Ardani IGAW, Wicaksono A, Hamid T. The Occlusal Plane Inclination Analysis for Determining Skeletal Class III Malocclusion Diagnosis. Clin Cosmet Investig Dent. 2020;12:163-171.
2. Masucci C, Oueiss A, Maniere-Ezvan A, Orthlieb JD, Casazza E. Qu'est-ce qu'une malocclusion ? [What is a malocclusion?]. Orthod Fr. 2020;91(1-2):57-67.
3. Guo L, Feng Y, Guo HG, Liu BW, Zhang Y. Consequences of orthodontic treatment in malocclusion patients: clinical and microbial effects in adults and children. 2016. BMC Oral Health. 28;16(1):112.
4. Badan Penelitian dan Pengembangan Kesehatan Kementerian Kesehatan RI. 2018 Hasil Utama Riset Kesehatan Dasar (Riskesdas).94.
5. Laguhi PA, Anindita PS, Gunawan PN. Gambaran maloklusi dengan menggunakan HMAR pada pasien di Rumah Sakit Gigi dan Mulut Universitas Sam Ratulangi Manado. Jurnal e-GiGi. 2014;2, 2: 2-6.
6. Zhang M, McGrath C, Hägg U. The impact of malocclusion and its treatment on quality of life: a literature review. Int J Paediatr Dent. 2006;16(6):381-7.
7. Azaripour A, Weusmann J, Mahmoodi B, Peppas D, Gerhold-Ay A, Van Noorden CJ, Willershausen B. Braces versus Invisalign®: gingival parameters and patients' satisfaction during treatment: a cross-sectional study. BMC Oral Health. 2015;15:69.
8. Asiry MA. Biological aspects of orthodontic tooth movement: A review of literature. Saudi J Biol Sci. 2018;25(6):1027-1032.
9. Henneman S, Von den Hoff JW, Maltha JC. Mechanobiology of tooth movement. Eur J Orthod. 2008;30(3):299-306.
10. Hass R, Kasper C, Böhm S, Jacobs R. Different populations and sources of human mesenchymal stem cells (MSC): A comparison of adult and neonatal tissue-derived MSC. Cell Commun Signal. 2011;9:12.
11. Motamedian SR, Tabatabaei FS, Akhlaghi F, Torshabi M, Gholamin P, Khojasteh A. Response of Dental Pulp Stem Cells to Synthetic, Allograft, and Xenograft Bone Scaffolds. Int J Periodontics Restorative Dent. 2017;37(1):49-59.
12. Tsichlaki A, Chin SY, Pandis N, Fleming PS. How long does treatment with fixed orthodontic appliances last? A systematic review. Am J Orthod Dentofacial Orthop. 2016;149(3):308-18.
13. Miles P. Accelerated orthodontic treatment - what's the evidence? Aust Dent J. 2017;62 Suppl 1:63-70.
14. Duan X, Tu Q, Zhang J, Ye J, Sommer C, Mostoslavsky G, Kaplan D, Yang P, Chen J. Application of induced pluripotent stem (iPS) cells in periodontal tissue regeneration. J Cell Physiol. 2011;226(1):150-7.
15. Motamedian SR, Iranparvar P, Nahvi G, Khojasteh A. Bone Tissue Engineering: A Literature Review. Regen Reconst Restor 2016;1:103-120.

16. Mafi R, Hindocha S, Mafi P, Griffin M, Khan WS. Sources of adult mesenchymal stem cells applicable for musculoskeletal applications - a systematic review of the literature. Open Orthop J. 2011;5 Suppl 2:242-8.
17. Hass R, Kasper C, Böhm S, Jacobs R. Different populations and sources of human mesenchymal stem cells (MSC): A comparison of adult and neonatal tissue-derived MSC. Cell Commun Signal. 2011;9:12.
18. Narmada IB, Laksono V, Nugraha AP, Ernawati DS, Winias S, Prahasanti C, Dinaryanti A, Susilowati H, Hendrianto E, Ihsan IS,Rantam FA. Regeneration of Salivary Gland Defects of Diabetic Wistar Rats Post Human Dental Pulp Stem Cells Intraglandular Transplantation on Acinar Cell Vacuolization and Interleukin-10 Serum Level. Pesquisa Brasileira em Odontopediatria e Clínica Integrada 2019; 19(e5002):1-10
19. Gay IC, Chen S, MacDougall M. Isolation and characterization of multipotent human periodontal ligament stem cells. Orthod Craniofac Res. 2007;10(3):149-60.
20. Khojasteh A, Motamedian SR, Rad MR, Shahriari MH, Nadjmi N. Polymeric vs hydroxyapatite-based scaffolds on dental pulp stem cell proliferation and differentiation. World J Stem Cells. 2015;7(10):1215-21.
21. Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, Shi S. SHED: stem cells from human exfoliated deciduous teeth. Proc Natl Acad Sci U S A. 2003;100(10):5807-12.
22. Saskianti T, Nugraha AP, Prahasanti C, Ernawati DS, Tanimoto K, Riawan W, Kanawa M, Kawamoto T, Fujimoto K. Study of Alveolar Bone Remodeling Using Deciduous Tooth Stem Cells and Hydroxyapatite by Vascular Endothelial Growth Factor Enhancement and Inhibition of Matrix Metalloproteinase-8 Expression in vivo. Clin Cosmet Investig Dent. 2022;14:71-78.
23. Wu Y, Chen L, Scott PG, Tredget EE. Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis. Stem Cells. 2007;25(10):2648-59.
24. Zainal Ariffin SH, Yamamoto Z, Zainol Abidin IZ, Megat Abdul Wahab R, Zainal Ariffin Z. Cellular and molecular changes in orthodontic tooth movement. ScientificWorldJournal. 2011;11:1788-803.
25. Feng L, Yang R, Liu D, Wang X, Song Y, Cao H, He D, Gan Y, Kou X, Zhou Y. PDL Progenitor-Mediated PDL Recovery Contributes to Orthodontic Relapse. J Dent Res. 2016;95(9):1049-56.
26. Safari S, Mahdian A, Motamedian SR. Applications of stem cells in orthodontics and dentofacial orthopedics: Current trends and future perspectives. World J Stem Cells. 2018;10(6):66-77.
27. Feng X, Teitelbaum SL. Osteoclasts: New Insights. Bone Res. 2013;1(1):11-26.
28. Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. Nature. 2003;423(6937):337-42.
29. Asagiri M, Takayanagi H. The molecular understanding of osteoclast differentiation. Bone. 2007;40(2):251-64.
30. Long F. Building strong bones: molecular regulation of the osteoblast lineage. Nat Rev Mol Cell Biol. 2011 ;13(1):27-38.
31. Karsenty G, Kronenberg HM, Settembre C. Genetic control of bone formation. Annu Rev Cell Dev Biol. 2009;25:629-48.
32. Blair HC, Larrouture QC, Li Y, Lin H, Beer-Stoltz D, Liu L, Tuan RS, Robinson LJ, Schlesinger PH, Nelson DJ. Osteoblast Differentiation and Bone Matrix Formation In Vivo and In Vitro. Tissue Eng Part B Rev. 2017;23(3):268-280.
33. Cummings SR, San Martin J, McClung MR, Siris ES, Eastell R, Reid IR, Delmas P, Zoog HB, Austin M, Wang A, Kutilek S, Adami S, Zanchetta J, Libanati C, Siddhanti S, Christiansen C; FREEDOM Trial. Denosumab for prevention of fractures in postmenopausal women with osteoporosis. N Engl J Med. 2009;361(8):756-65.
34. Nareswari RAAR, Narmada IB, Djaharu'ddin I, Rahmawati D, Putranti NAR, Nugraha AP. Effect of vitamin D administration on vascular endothelial growth factor expression and angiogenesis number in orthodontic tooth movement of pregnant Wistar rats. v 2019; 33(3): 182-8.
35. Nugraha AP, Narmada IB, Ernawati DS, Widodo DWW, Lestari P, Dinaryanti A, Hendrianto E, Ihsan IS, Susilowati H. Gingival Mesenchymal Stem Cells from Wistar Rat's Gingiva (Rattus Novergicus) – Isolation and Characterization (In Vitro Study). J Int Dent Med Res 2018; 11(2):694-699.
36. Nugraha AP, Rantam FA, Narmada IB, Ernawati DS, Ihsan IS. Gingival-Derived Mesenchymal Stem Cell from Rabbit (Oryctolagus cuniculus): Isolation, Culture, and Characterization. Eur J Dent. 2021;15(2):332-339.
37. Nugraha AP, Ihsan IS, Dinaryanti A, Hendrianto E, Susilowati H, Prasetyo EP, Narmada IB, Ernawati DS, Nugraha AP, Kharisma VD, Riawan W, Rantam FA. Cobalt (II) Chloride in Enhancing Hypoxia Inducible Factor-1α Expression of Gingival Derived Mesenchymal Stem Cells in Vitro. Research Journal of Pharmacy and Technology. 2021; 14(5):2639-2
38. Hisham PBBM, Narmada IB, Alida A, Rahmawati D, Nugraha AP, Putranti NA. Effects of Vitamin D in Alveolar Bone Remodeling on Osteoblast Numbers and Bone Alkaline Phosphatase Expression in Pregnant Rats During Orthodontic Tooth Movement. J Orofac Sci 2019;11:79-83
39. Narmada IB, Husodo KRD, Ardani IGAW, Rahmawati D, Nugraha AP, Iskandar RPD. Effect of Vitamin D during Orthodontic Tooth Movement on Receptor Activator of Nuclear Factor Kappa-B Ligand Expression and Osteoclast Number in Pregnant Wistar Rat (Rattus novergicus). JKIMSU 2019;8(1): 38-42.
40. Syarif RD, Kusumaningsih T, Arundina I. Changes in osteoblast and osteoclast cell count after moringa oleifera leaf extract administration during orthodontic tooth movement. Journal of Dentomaxillofacial Science. 2020;5(2): 98-102.
41. Ismardianita W, Nasrul W, Yanwirasti. Effect of ethanol extract of myrmecodia pendens on TGF-β1 expression and osteoblast cells after tooth extraction (experimental research on cavia cobaya). J Dentomaxillofac Sci. 2017;2: 150-154.
42. Rucci N. Molecular biology of bone remodelling. Clin Cases Miner Bone Metab 2008;5:49-56.
43. Despande D. Autologous Osteoblast-cell Therapy in Orthodontics and Implantology: A Single-center Experience of Nine Patients. Int J Oral Implantol Clin Res. 2018;9(1–3): 55–59.
44. Feller L, Khammissa RA, Schechter I, Thomadakis G, Fourie J, Lemmer J. Biological Events in Periodontal Ligament and Alveolar Bone Associated with Application of Orthodontic Forces. ScientificWorldJournal. 2015;2015:876509.
45. Nugraha AP, Narmada IB, Ernawati DS, Dinaryanti A, Hendrianto E, Ihsan IS, Riawan W, Rant am FA. Osteogenic potential of gingival stromal progenitor cells cultured in platelet rich fibrin is predicted by core-binding factor subunit-α1/Sox9 expression ratio (in vitro). F1000Research 2018, 7:1134.
46. Nugraha AP, Narmada IB, Ernawati DS, Dinaryanti A, Hendrianto E, Riawan W, Rantam FA. Bone alkaline phosphatase and osteocalcin expression of rat's Gingival mesenchymal stem cells cultured in platelet-rich fibrin for bone remodeling (in vitro study). Eur J Dent 2018;12:566-7.
47. Nugraha AP, Narmada IB, Ernawatı DS, Dınaryantı A, Hendrıanto E, Ihsan IS, Riawan W, Rantam FA. The aggrecan expression post platelet rich fibrin administration in gingival medicinal signaling cells in Wistar rats (Rattus novergicus) during the early osteogenic differentiation (in vitro). Kafkas Univ Vet Fak Derg, 2019;25 (3): 421-425, 2019.
48. Nugraha AP, Narmada IB, Ernawati DS, Dinaryanti A, Susilowati H, Hendrianto E, Ihsan IS, Riawan W, Rantam FA. Somatic Cells Acceleration by Platelet Rich Fibrin. Indian Vet J 2019;96(04):30-34.
49. Menicanin D, Mrozik KM, Wada N, Marino V, Shi S, Bartold PM, Gronthos S. Periodontal-ligament-derived stem cells exhibit the capacity for long-term survival, self-renewal, and regeneration of multiple tissue types in vivo. Stem Cells Dev. 2014 May 1;23(9):1001-11.
50. Bartold M, Gronthos S, Haynes D, Ivanovski S. Mesenchymal stem cells and biologic factors leading to bone formation. J Clin Periodontol. 2019;46(Suppl 21):12-32.
51. Fawzy El-Sayed KM, Dörfer CE. Gingival Mesenchymal Stem/Progenitor Cells: A Unique Tissue Engineering Gem. Stem Cells Int. 2016;2016:7154327.
52. Fawzy El-Sayed KM, Dörfer CE. Gingival Mesenchymal Stem/Progenitor Cells: A Unique Tissue Engineering Gem. Stem Cells Int. 2016;2016:7154327.
53. Nugraha AP, Narmada IB, Ernawati DS, Dinaryanti A, Hendrianto E, Riawan W, Rantam FA. Bone alkaline phosphatase and osteocalcin expression of rat's Gingival mesenchymal stem cells cultured in platelet-rich fibrin for bone remodeling (in vitro study). Eur J Dent. 2018;12:566-7.
54. Robinson JA, Chatterjee-Kishore M, Yaworsky PJ, Cullen DM, Zhao W, Li C, Kharode Y, Sauter L, Babij P, Brown EL, Hill AA, Akhter MP, Johnson ML, Recker RR, Komm BS, Bex FJ. Wnt/beta-catenin signaling is a normal physiological response to mechanical loading in bone. J Biol Chem. 2006;281(42):31720-8.
55. Yamaguchi Y, Passeron T, Watabe H, Yasumoto K, Rouzaud F, Hoashi T, Hearing VJ. The effects of dickkopf 1 on gene expression and Wnt signaling by melanocytes: mechanisms underlying its suppression of melanocyte function and proliferation. J Invest Dermatol. 2007;127(5):1217-25.
56. Di Domenico M, D'apuzzo F, Feola A, Cito L, Monsurrò A, Pierantoni GM, Berrino L, De Rosa A, Polimeni A, Perillo L. Cytokines and VEGF induction in orthodontic movement in animal models. J Biomed Biotechnol. 2012;2012:201689.
57. Niklas A, Proff P, Gosau M, Römer P. The role of hypoxia in orthodontic tooth movement. Int J Dent. 2013;2013:841840.
58. Knothe Tate ML. "Whither flows the fluid in bone?" An osteocyte's perspective. J Biomech. 2003;36(10):1409-24.
59. Zhang P, Wu Y, Jiang Z, Jiang L, Fang B. Osteogenic response of mesenchymal stem cells to continuous mechanical strain is dependent on ERK1/2-Runx2 signaling. Int J Mol Med. 2012;29(6):1083-9.
60. Yamamoto K, Yamamoto T, Ichioka H, Akamatsu Y, Oseko F, Mazda O, Imanishi J, Kanamura N, Kita M. Effects of mechanical stress on cytokine production in mandible-derived osteoblasts. Oral Dis. 2011;17(7):712-9.
61. Zhu J, Zhang X, Wang C, Peng X, Zhang X. Different magnitudes of tensile strain induce human osteoblasts differentiation associated with the activation of ERK1/2 phosphorylation. Int J Mol Sci. 2008;9(12):2322-32.
62. Tuncay OC, Ho D, Barker MK. Oxygen tension regulates osteoblast function. Am J Orthod Dentofacial Orthop. 1994;105(5):457-63.
63. Mao Y, Wang L, Zhu Y, Liu Y, Dai H, Zhou J, Geng D, Wang L, Ji Y. Tension force-induced bone formation in orthodontic tooth movement via modulation of the GSK-3β/β-catenin signaling pathway. J Mol Histol. 2018;49(1):75-84.
64. Hermawan RW, Narmada IB, Djaharu'ddin I, Nugraha AP, Rahmawati D. 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. Research J. Pharm. and Tech. 2020; 13(4):1730-1734
65. Nugraha AP, Narmada IB, Sitasari PI, Inayati F, Wira R, Triwardhani A, et al. 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. 2020; 20:e5347.
66. Sharaf-Eldin WE, Abu-Shahba N, Mahmoud M, El-Badri N. The Modulatory Effects of Mesenchymal Stem Cells on Osteoclastogenesis. Stem Cells Int. 2016;2016:1908365.
67. Sumi K, Abe T, Kunimatsu R, Oki N, Tsuka Y, Awada T, Nakajima K, Ando K, Tanimoto K. The effect of mesenchymal stem cells on chemotaxis of osteoclast precursor cells. J Oral Sci. 2018;60(2):221-225.