The Differences of Effectiveness HBO 2,4 ATA between 7 and 10 Days in Bone Remodelling of Tension Area of Orthodontic Tooth Movement

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Abstract. Mechanical force of orthodontics, would inhibit periodontal ligament vascularization and blood flow, causing biochemical and cellular changes, such as cell deformation, inflammation, and circulatory disturbances. Each of these conditions affecting cell differentiation, cell repair, and cell migration, is driven by numerous molecular and inflammatory mediators. Fibroblasts, osteoblasts, osteocytes, osteoclasts, odontoblasts, cementoblasts, chondrocytes and immune cells are the major cell types involved in the remodeling process on orthodontic tooth movement. Hyperbaric oxygen therapy is one of many solutions which stimulates the growth of new blood vessels and result in a substantial increase in tissue oxygenation. It plays a role in bone remodeling process. Purpose: To determine the differences of Hyperbaric Oxygen (HBO)2,4 ATA 7 and 10 days in osteoblast and osteocytes number during bone remodelling in Orthodontic tooth movement. Materials and Methods: The experiment using a post test only control group design. 32 male guinea pigs were randomly divided into 4 groups. K1 was control group without any treatment, K2 was a group which was given a mechanical orthodontic pressure, K3 was the group treated mechanical orthodontic with the addition of hyperbaric oxygen therapy. The maxillary incisors were moved distally by elastic separator. After HBO on day 7, all groups were sacrificed then analyzed osteoblast and osteocytes number by One-way ANOVA and LSD statistical test. Results: The study showed significant differences in osteoblast and osteocytes number during bone remodelling in orthodontic tooth movement between groups. Conclusion: HBO therapy 2.4 ATA for 7 days effective to induce bone remodelling during orthodontic tooth movement.

Keywords: Osteoblast, Osteocytes, Orthodontic Tooth, Bone Remodelling.

1. Introduction
Orthodontic treatment aims to correct malocclusion based on the biological properties of bone tissue. If a tooth is given a force, the power then will be transmitted to the tissue supporting the tooth, triggering a reaction inside the periodontal tissue and alveolar bone [1]. Orthodontic tooth movement, moreover, is considered as a combination of bone resorption and bone apposition in pressure and tension areas. Orthodontic force can inhibit periodontal ligament vascularization and blood flow, causing biochemical and cellular changes as well as alveolar bone contour changes. Therefore, bone remodeling that occurs during orthodontic tooth movement is a biological process involving an acute inflammatory response to periodontal tissue. A histological research shows that the first stage of resorption occurs in 3-5 days followed by recovery process in 5-7 days. This is then followed by the final stage of bone remodeling between 7 and 14 days [2, 3].

Thus, optimal orthodontic force is needed to move teeth into the determined direction without causing discomfort to the patients as well as tissue damage [4]. The optimal force for moving a tooth is the lightest force that can cause maximum response. However, the threshold of the force for tooth movement is very low and different for each tooth. The response of tissues and supporting teeth to the force is also known to depend on the size of the force [5].
Bone remodeling, further more, requires a long process, and usually leads to complications, especially when pressure strength given is too strong to cause blood vessels to be closed and nutrient flow to be blocked. Consequently, there will be a change in the direction of tissue regression, periodontium cell and fiber disappearance, as well as hyaline degeneration experience so that resorption does not occur directly. In other words, it takes various efforts to accelerate tooth movement process, one of which is hyperbaric oxygen therapy. Hence, the effects of hyperbaric oxygen therapy on the process of bone formation need to be studied and assessed.

Bone is a hard tissue consisted of several main components. First, extracellular matrix is mainly consisted of type I collagen and various bone-specific proteins. Second, inorganic minerals (67% of the bone) are consisted of calcium and phosphate in the form of hydroxyapatite crystals. Third, osteoblasts play a role in bone matrix mineralization. Fourth, osteocytes and osteoclasts as multinucleate cells are derived from circulating hematopoietic precursors that function for bone resorption [6].

Besides, osteoblasts play a role in activating osteoclasts through the formation of various cytokines as well as in regulating bone homeostasis [7]. Osteoblasts also play a role in synthesizing collagens to form osteoids as bone base materials. In the remodeling process, osteoblasts will disaggregate bone intercellular substances containing collagens to synthesize new collagen fibers and form osteoid [8]. Osteoblasts surrounded by bone minerals then differentiate into osteocytes. Osteocytes binding to one another through cytoplasmic extension in the bone canaliculi are thought to be responsible for detecting the presence of forces affecting the bone [4].

On the other hand, oxygen is considered as an important element in the process of callus formation during bone remodeling process. Oxygen in hyperbaric conditions has several effects. First, it can eliminate free radicals after tooth movement (hematoma phase) so that tissue death can be reduced. Second, it can stimulate the improvement of damaged blood vessels (neovascularization). Third, it can increase osteoblast activity in bone formation (osteogenesis). Fourth, at the high level, it can prevent edema and swelling caused by vascular vasoconstriction (small) during the inflammatory phase. Fifth, it can maintain angiogenesis in remodeling process [9].

Hyperbaric Oxygen Therapy (HBO) is a method of treatment using pure oxygen (100%) with air pressure greater than normal atmospheric pressure. Hyperbaric oxygen treatment has an effect on oxygen delivery, accelerating 2 to 3 times greater than in ordinary atmosphere. HBO therapy can also deliver oxygen quickly and systemically with high concentrations to wound areas. Increased pressure will change the normal respiration process in cells and cause oxygen to dissolve in the plasma. HBO therapy, therefore, is beneficial because it stimulates the growth of new blood vessels and results in a substantial increase in tissue oxygenation that can capture several types of infections, and improve wound healing. As an adjuvant therapy, HBO is suitable for use in a number of surgical conditions. The following mechanisms have been identified to improve the healing process of certain conditions, such as hyper-oxygenation, vasoconstriction, bactericidal or bacteriostatics, angiogenesis, neovascularization, and direct pressure [9].

In addition, orthodontic treatment has an average duration of about 15-24 months, so various methods can be used to speed up orthodontic treatment duration. For instance, the administration of hyperbaric oxygen therapy stimulates the formation of new blood vessels (neovascularization), thus stimulating the remodeling process by increasing osteoblast activity. In a previous research, the administration of hyperbaric oxygen at 2.4 ATA (90 minutes a day) for 7 days can increase trabecular bone volume number indicating osteoblast activity during tooth movement in rats [9]. As a result, based on those literature studies or references and previous researches, this research aimed to compare the effectiveness of seven day and ten day-hyperbaric oxygen therapies at 2.4 ATA (90 minutes a day) on the remodeling process during tooth movement by observing the number of osteoblasts and osteocytes as the basic ingredients of bone formation.

2. Experimental Method
2.1 Place of experiment
This research was a true experimental laboratory study with Post Test Only Control Group Design. This research was performed at: 1) Biochemistry Laboratory, Faculty of Medicine, Universitas Airlangga - Surabaya; 2) Marine Health Institute of Dr.Ramelan Hospital - Surabaya; and 3) Anatomy Pathology Laboratory of Dr.Sutomo Hospital - Surabaya.
2.2 Material and Instrument
Experimental animals used in this research were thirty-two male Cavia cobayas aged 3-4 months and weighed 350-500 grams. And, materials used were 100% pure oxygen in animal hyperbaric chamber, separator, Ketamine 10% with a dose of 0.1 ml / kg IM BM, betadine solution, cotton, husk, guinea pig food, aquades, wire woven cage sized 17 x 34 x 34 cm, plastic cage sized 60 x 40 x 20 cm (for displacement), 2 cc syringe, Force Module Separator, sterile scalpel and handle, surgical scissors, reaction glasses, scales, Rotary microtome, as well as microscope.

2.3 Management of Experiment
Furthermore, those thirty-two male Cavia Cobayas were divided into four groups, namely Group (-) as negative control group, Group (+) as positive control group, Group 1 as treatment group 1, and Group 2 as treatment group 2. Every group was consisted of eight animals put in a cage (sized 60x40x34 cm), given chaff, and covered with woven wire. Those animals were also fed with a lot of crude fiber, corn tubers, and other greens in adlibitium. Next, the cages were placed in a quiet room at room temperature, not directly exposed to sunlight, but with adequate lighting. Those animals then were adapted for 24 hours before given treatment. In Group (+), Group P1, and Group P2, separators were set on their maxillary incisors before the animals were anesthetized with Ketamine 10% with a dose of 0.1ml / kg BW IM. Hyperbaric oxygen therapy then was given (in the chamber) to Groups 1 for 7 days and 10 days to Group 2 without removing the separators.

After the animals in Group 1 and Group 2 were put into the animal chamber, the pressure in chamber was set up to 2.4 ATA. Pure oxygen (100%) then was flowed for 3x30 minutes. Afterwards, the pressure was stopped and lowered to its original level (1 ATA). The animals then were put out the chamber into their original cages. The treatment was carried out from day 1 to day 7 for Group 1, while for Group 2 it was conducted until day 10.

On the 7th day after the administration of hyperbaric oxygen, the animals in Group (-), Group (+), and Group 1 were anesthetized with overdose (Overdose of Chemical Anesthetics) and then decapitated for taking their maxillary jaws. Meanwhile, those animals in Group 2 were sacrificed on the 10th day. Afterwards, the maxillary jaws were fixed in a buffered formalin solution and softened in EDTA solution. Those animals that had been decapitated were then buried.

Subsequently, maxillary jaws which had been fixed in buffered formalin solution and softened in EDTA solution were given to the Anatomical Pathology Laboratory of Dr. Sutomo Hospital – Surabaya to make preparations using Hematoxylin Eosin (HE) staining. They then were observed using a microscope to make photograph. After that, the number of osteoblasts, osteoclasts, osteocytes, and fibroblasts were calculated using the microscope with a magnification of 400x. One preparation was calculated three times at different fields of view.

3. Result
The data of osteoblasts collected in this research were analyzed using descriptive statistics to reveal the distribution of the data as well as to present the data clearly as illustrated in table 1. below:

| Table 1. Results of the descriptive statistics |
|-----------------------------------------------|
| Groups | N  | Mean± Standard Deviation |
| Group (-) | 8  | 3.142 ± 1.573 |
| Group (+) | 8  | 4.833 ± 1.602 |
| Group 1  | 8  | 14.571 ± 6.320 |
| Group 2  | 8  | 18.166 ± 6.615 |
| Total    | 32 |                         |
Next, the normality of the data in the negative control group, the positive control group, the treatment group 1, and the treatment group 2 was analyzed using Shapiro-Wilk test since the samples used were less than 50. Results of the Shapiro-Wilk test were depicted in the following table:

Table 2. Results of the normality test

| Variables   | Shapiro – Wilk | Sig  |
|-------------|----------------|------|
| Group (-)   | 0.932          | 0.570*|
| Group (+)   | 0.809          | 0.070*|
| Group 1     | 0.847          | 0.116*|
| Group 2     | 0.882          | 0.278*|

Table 2 above demonstrated that the results of the Shapiro-Wilk test of all groups had a significant value of p> 0.05. It indicates that the differences of osteoblasts in the groups given hyperbaric oxygen therapy for 7 and 10 days during the tooth movement were normally distributed.

Table 3. Results of the Levene test

| Levene Test | Sig  |
|-------------|------|
| 2.029       | 0.139*|

Afterwards, the homogeneity of the data was analyzed using a parametric test, one way ANOVA test, to determine the differences of osteoblasts between the groups given hyperbaric oxygen therapy for 7 and 10 days during the tooth movement. Results of the one way ANOVA test showed that there was no difference in variance of osteoblasts between all groups as follow:

Table 4. Results of one way ANOVA test

| Variance                  | P    |
|---------------------------|------|
| between groups            | 0.000|
| between treatment groups  |      |
| Total                     |      |

In table 4, the significance value obtained was 0.000 (p<0.05). It suggests that there was a significant difference between the negative group and the positive group with each treatment group having different treatments. Thus, LSD test was performed to find out which groups had differences.
LSD test is a follow-up test used after knowing that there is a significant difference based on the results of one way ANOVA test. In this research, LSD test was conducted to see the differences of osteoblasts among the groups. Two treatment groups can be said to be significantly different if the significant value of the LSD test is less than the research error level of 0.05 (5%). On the contrary, if the significant value of the LSD test is greater than 0.05 (5%), there will be no significant difference between the groups.

Table 5. Results of LSD test

| Mean Groups | K- (3.142) | K+ (4.833) | K1 (14.571) | K2 (18.166) |
|-------------|------------|------------|-------------|-------------|
| Group (-) (3.142) | 0.049* | 0.000* | 0.000* | |
| Group (+) (4.833) | | 0.008* | 0.003* | |
| Group 1 (14.571) | | | | 0.559 |
| Group 2 (18.166) | | | | |

Note: *significant difference

Based on the results of the LSD test above, there were significant differences in the number of osteoblasts between Group K- and Group K+ (p 0.049), between Group K- and Group K1 (p 0.000), between Group K- and Group K2 (p 0.000), between Group K+ and Group K1 (p 0.008), as well as between Group K+ and Group K2 (p 0.003). Meanwhile, there was no significant difference between Group K1 and Group K2. In addition, the data of osteocytes collected were also analyzed descriptively to reveal the distribution of the data as well as to present the data clearly.

Table 6. Results of the descriptive statistical test on the number of osteocytes in the tension areas

| Treatment | n | Mean | Standard Deviation |
|-----------|---|------|--------------------|
| Group (-) | 8 | 28.8 | 7.6                |
| Group (+) | 8 | 29.4 | 7.2                |
| Group 1   | 8 | 50.16| 16.96             |
| Group 2   | 8 | 75.5 | 11.30             |

Figure 2. Osteocytes in tension area, magnification 40X
Results of the Shapiro-Wilk test showed that the data of osteocytes in all groups were normally distributed. Results of the Levene test then indicated a significance value of 0.14. It means that the data of osteocytes were homogeneous (p>0.05). Since the data of osteocytes were normally distributed and homogeneous, a parametric test, one way ANOVA test, was carried out to determine the differences in the number of osteocytes between the negative control and the positive control group with all treatment groups given hyperbaric oxygen therapy.

| Variance                  | P     |
|---------------------------|-------|
| between the treatment groups| 0.000 |
| Total                     |       |

Results of the one way ANOVA test indicated a significance value of 0.000 (p<0.05). This suggests that there was a significant difference in the number of osteocytes between the negative control and each treatment group. Therefore, LSD test was performed.

| (I) Treatment | (J) Treatment | Mean (I) | Mean (J) | Sig.  |
|---------------|---------------|----------|----------|-------|
| Group (-)     | Group 1       | 28.8     | 50.16    | 0.005*|
|               | Group 2       |          | 75.5     | 0.000*|
| Group 1       | Group 2       | 50.16    | 75.5     | 0.002*|

Results of the LSD test revealed that there were significant differences between Group K2 and Group K (-), between Group K2 and Group K1, as well as between Group K1 and Group K (-).

### 4. Discussion

This research aimed to determine differences in the number of osteoblasts and osteocytes located in the maxillary bones of those Cavia cobayas during tooth movement treated hyperbaric oxygen therapy between for 7 and 10 days. Hence, the samples used in this research were thirty-two Cavia cobayas. Those kind animals were selected since they are the easiest ones to take care and control in laboratory [13]. Besides, they are very suitable for studying orthodontic tooth movement. They are also relatively inexpensive, and their histological preparation is easier than other animals’[14]. They then were divided into four groups, namely negative control group without treatment; positive control group only set with separator; Group 1 treated with HBO 2.4 ATA therapy for 7 days; and Group 2 treated with HBO 2.4 ATA therapy for 10 days.

Variables of the HBO therapy used in this research, moreover, refer to those suggested in a previous research conducted by Lakesla-RSAL, Surabaya namely HBO therapy 2.4 ATA using O2 100% for 3x30 minutes with 5 minute interval to breathe, carried out every day for 10 consecutive days [12]. In general, the administration of HBO therapy is actually between 90 to 120 minutes with pure oxygen at 2.0 - 2.5 ATA. The administration of HBO therapy for 7 days is actually based on several experimental findings [9,10,15]. First, it is known that the administration of hyperbaric oxygen therapy for 7 days during tooth movement can increase trabecular bone volume and trabecular bone number, indicated by the number of osteoblast activities [9]. Second, there is also a significant difference in the number of osteoblasts between the group treated with hyperbaric oxygen therapy for 7 days and that without hyperbaric oxygen therapy [10]. This is because oxygen is an important element in callus formation during bone remodeling since it can improve osteoblast activity in bone formation (osteogenesis) [9].

Furthermore, the results of the descriptive statistical analysis using one way ANOVA test and LSD difference test revealed that there were an increase in the mean number of the cells as well as significant...
differences in the number of the cells between Group 1 (HBO 7 days) and Group 2 (HBO 10 days) with the negative control group (without treatment) and the positive control group (only with separator). It means that the presence of a separator or orthodontic force will result in changes in tissue around the teeth, and then make the teeth move managed in pressure and tension areas [16]. Orthodontic force in pressure area can immediately cause bone resorption in the area. Meanwhile, orthodontic force in tension area can make teeth move away from the alveolar wall, leading to bone apposition in the area. Cells participating in those processes are osteoblasts [17].

In general, bone formation process due to mechanical stress will occur in two reactions, namely local reaction, including biological electricity reaction, blood flow, micro-fractures, producing prostaglandins, cytokines, and cyclic adenosine monophosphate (cAMP), and systemic reaction involving the activities of parathyroid hormone, vitamin D, and calcitonin. The combination of these two reactions then can generate both osteoblasts on the tension areas playing a role in apposition process, and osteoclasts on the pressure areas playing a role in resorption process. Osteoclasts and osteoblasts are the two main cell types found in bone as the main producers of bone materials [16].

The function and activation of osteoblasts are caused by growth factors, such as parathyroid hormone and cytokines, prostaglandin E2 (PGE2). Parathyroid hormone increases calcium flow and maintains the body's extracellular calcium levels at a relatively constant level. Osteoblasts are the only bone cells that have parathyroid hormone receptors. This hormone can cause cytoskeletal changes in osteoblasts.

On the other hand, there are two main growth factors needed for the functional formation of osteoclasts, namely Macrophage-Colony Stimulating Factor (M-CSF) and RANKL, expressed by osteoblasts in response to resorptive stimuli by parathyroid hormone [20]. Macrophage-Colony Stimulating Factor (M-CSF) plays an important role in the proliferation, survival, and activation of osteoclast precursors. Macrophage-Colony Stimulating Factor is secreted by a number of cells including osteoblasts, fibroblasts, monocytes and endothelial cells [21]. Macrophage-Colony Stimulating Factor induces RANK expression (receptor for RANKL) [22].

The interaction of RANKL or RANK is modified by other factors produced by osteoblasts, called as osteoprotegerin (OPG), a soluble feed receptor for RANKL [23]. Osteoprotegerin prevents RANK from binding to RANK, so OPG suppresses bone resorption and maintains a balance between bone resorption and bone formation.

HBO therapy stimulates monocytes, fibroblast function, and collagen synthesis, as well as increases vascular density [27]. HBO therapy increases the local concentration of reactive nitrogen species (RNS) and reactive oxygen species (ROS) which can affect osteoclast differentiation and activity, as well as regulate other critical aspects of bone metabolism. Reactive oxygen species increase expression of RANKL [28], alter the ratio of RANKL or osteoprotegerin, and help osteoclast differentiation. HBO therapy generating ROS and RNS also induces stem cell mobilization and vasculogenesis, reducing areas of slight vascularization in bone and improving remodeling the necrotic area [29].

The main damage caused by ROS is change in macromolecules, such as fatty acid polyunsaturation in membrane lipids, essential proteins, and DNA. Excessive Oxygen Species can also interfere with cell function, including beta cells, endothelial cells, fat, muscle and nerve cells [30]. ROS and other free radicals are produced during HBO therapy. In other words, this procedure can trigger the activity of some free radicals. HBO treatment pressure, according to Ozden, never exceeds 3 ATA and usually does not last longer than 90 minutes. Nevertheless, the effects of HBO treatment still can be countered by certain enzymes responsible for lipid peroxidation, such as superoxide dismutase. Free radicals in the tissue will be offset by superoxide dismutase (SOD) to prevent tissue injury as an antioxidant defense system. Thus, it can be said that HBO treatment still can trigger an antioxidant mechanism and reduce oxidative stress [31,32].

Osteoblasts play a role in the synthesis of bone matrix organic components, namely type I collagen, proteoglycans, and glycoproteins including osteonectin [33]. Immature osteoblasts with high levels of osteopontin can also differentiate into mature osteoblasts with high-level osteocalcin. The mature osteoblasts embedded in the bone matrix then will become osteocytes [36].

Bone resorption and bone formation actually occurs at the same time. Osteoblasts work only in places where osteoclasts have completed resorption. On the pathway, some factors released from resorbed bone or local increases due to mechanical stimuli by bone resorption can stimulate precursor cells to osteoblast proliferation and differentiation [37].
Subsequently, osteocytes produce nitric oxide (NO), prostaglandin, and tumor necrosis factor α (TNF-α). Nitric oxide (NO) is an important regulator of bone responses to mechanical stress. NO is produced through constitutive endothelial nitric oxide synthase (eNOS) activity or nitric oxide synthase (NOS). Several in vitro researches have shown that NO rapidly increases the response to mechanical stress in bone cells. NO also plays a role in bone formation, protects it against osteocyte apoptosis, and reduces osteoclast activity. Thus, an increase in NO levels can eliminate osteoclast activity, whereas inhibition of NO production can accelerate osteoclastogenesis and osteoclast activity [2,16].

Osteocytes, most commonly found mature bones, are derived from osteoblasts and located in the bone matrix. Manolagas argues that osteoblast producing matrix can become osteocytes [17]. Osteocytes have long cytoplasmic extensions that cover a large surface area to perform a multipurpose role in bone biology, including detection of changes in mechanical loads [4].

Oxygen is an important element in the process of callus formation during bone remodeling [9]. In orthodontic treatment, bone remodeling occurs in the alveolar bone and periodontal ligament. Bone remodeling involves both bone apposition triggered by osteoblasts and bone resorption by osteoclasts. [38]. Oxygen pressure plays a role in stimulating bone remodeling. An increase in oxygen pressure causes cellular differentiation into osseous tissue, while a decrease in oxygen pressure results in cartilage formation. There is parallelism between increased osteoblastic and osteoclastic pressures [9].

HBO therapy can accelerate osteoblast differentiation, improve the initial stage of mineralization, and cause a more obvious effect than hyperoxia or pressure alone. HBO therapy can also improves bone nodule formation and alkaline phosphatase activity in human osteoblasts. Alkaline phosphatase is a surface protein that can participate in the regulation of osteoblastic cell proliferation, migration and nodule formation and alkaline phosphatase activity. There is parallelism between increased osteoblastic and osteoclastic activities [9].

Hyperbaric oxygen therapy usually involving the administration of 100% oxygen in the atmosphere with greater pressure than absolute atmosphere (ATA) has been proposed as an adjunctive therapy to improve patient outcomes with fractures, osteoradionecrosis, and osteogenesis disorders, as well as those using bone graft and dental implant. A previous research on the use of hyperbaric oxygen therapy in animals even argues that this therapy can be used to treat fracture or nonunion fracture [41].

Hyperbaric oxygen therapy also serves to increase the concentration of oxygen in all body tissues, even in reduced blood flow, stimulate the growth of new blood vessels to increase blood flow in the reduced circulation, and trigger a widening of the arterial rebound to increase blood vessel dilation [42]. The blood vessels themselves play an important role in the administration of oxygen, nutrients, and other materials that are important for bone synthesis as well as considered as the source of osteoblast cells [17].

The procedure for administering HBO at 2-3 ATA with intermittent O2 will prevent O2 from poisoning [43]. This is because if you are in a pressure chamber (hyperbaric chamber) and pressed to 2.4 ATA, the partial arterial pressure (PO2) will increase 10 times so that the oxygen concentration in the blood will increase 10 times than normal. All body fluids (blood, lymph, and cerebrospinal) will also run very fast, and oxygen can reach damaged bones and soft tissues that cannot be entered by red blood cells, thus improving the function of white blood cells, increasing the formation of new capillaries (neovascularisari) and peripheral blood vessels, resulting in a rapid healing process [9].

Besides, in hyperbaric oxygen therapy, oxygen in the blood is transported in both a soluble form in plasma fluid and hemoglobin bond. However, there is only a small portion (3%) found in the soluble form. Actually, oxygen in this soluble form plays a very important role in this therapy since the soluble oxygen properties are more easily consumed by tissue through direct diffusion than oxygen bound to hemoglobin.12 The administration of hyperbaric oxygen therapy, consequently, can counteract the effects of hypoxia on the injured tissue and improve the quality of the tissue. In a hypoxic environment, the rate of bone resorption exceeds its apposition rate since the multipotent mesenchymal cells in the marrow fail to differentiate into osteoblasts [44].

The number of osteoblasts and osteocytes in the group with the administration of hyperbaric oxygen therapy for 7 days was not significantly different from those in the group with the administration of hyperbaric oxygen therapy for 10 days because of the adaptive response of the cells. The main physiological benefits of the adaptive response are to protect or maintain cells and organisms from high doses of toxic substances. The adaptive response is actually induced by oxidative stress since cells have two main defenses, namely antioxidant enzymes, such as superoxide dismutase (SOD), glutathione
peroxidase, and catalase directly involved in preventing oxidative cell damage, as well as repair enzymes eliminating or repairing damaged macromolecules oxidatively [45]. Hence, the hypothesis in this research was not proved.

In addition, another previous research conducted by Dong Wu (2007) also reveals how hyperbaric oxygen therapy affects the cellular mechanism of healing fractures, especially on the proliferation and differentiation of human osteoblasts in vitro, using laboratory-scale hyperbaric units. In this previous research, cell proliferation was evaluated daily by WST-1 assay for 10 consecutive days. On days 8 and 10, he found that there was no difference in the number of cells between a group given hyperbaric oxygen therapy and one without hyperbaric oxygen therapy. In other words, this previous research suggests that there is no significant change in cell membrane integrity before or after hyperbaric oxygen therapy between on day 8 and day 10 [41]. Finally, although this research was carried out on experimental animals, the administration of hyperbaric oxygen therapy at 2.4 ATA is still expected to be considered as an alternative therapy for orthodontic treatment to accelerate the process of bone remodeling in humans [10].

5. Conclusion
In conclusion, the administration of hyperbaric oxygen therapy at 2.4 ATA for 7 days and 10 days is considered to be effective in elevating in the number of osteoblasts. The administration of hyperbaric oxygen therapy at 2.4 ATA for 10 days can actually increase the number of osteoblasts more than the hyperbaric oxygen therapy for 7 days. However, it is not significantly different. Therefore, the administration of hyperbaric oxygen therapy at 2.4 ATA for 7 days is relatively good enough to increase vascularity in tissues. Similarly, the administration of hyperbaric oxygen therapy at 2.4 ATA can also generate the number of osteocytes in male Cavia cobayas during tooth movement since it can elevate cytokines and hormones that are important for tooth movement and bone formation.

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