

The changes of fibroblast and periodontal ligament characteristics in orthodontic tooth movement with adjuvant HBOT and propolis: A study in Guinea pigs

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ABSTRACT

Introduction: Periodontal ligament plays an essential role in preventing relapse after orthodontic treatment. Hyperbaric Oxygen Therapy (HBOT) and propolis gel can increase the amount of fibroblast in the tension area during orthodontic treatment, thus affecting the periodontal ligament. This research was aimed to analyse the difference of the width of the periodontal ligament and amount of fibroblast in the tension area with the administration of propolis gel and HBOT in an attempt to prevent orthodontic relapse. **Methods:** Forty-two male guinea pigs were randomly divided into 7 groups of treatments – the untreated group (I - negative group), group with rubber separator (II - positive group), 3% propolis gel treatment group (III), 5% propolis gel treatment group (IV), the HBOT treatment group (V), combination of 3% propolis gel and HBOT treatment group, and combination of 5% propolis gel and HBOT treatment group. The upper left central incisor was extracted distally using a 14-days separator rubber in the positive group and the treatment group; then the separator rubber was removed for 2 days to conduct the relapse process. The data were analysed by LSD statistical test. **Results:** The result of the combination of HBOT and propolis gel treatment showed significant differences among all groups ($p < 0.05$) in the width of the periodontal ligament (1.03), and the number of fibroblasts was 95.67 in the tension site. **Conclusions:** The combination of HBOT and propolis gel affect the width of periodontal ligament and the number of fibroblasts in the area of the orthodontic relapse.

Keywords: Fibroblast, periodontal ligament, orthodontic tooth movement, HBOT, propolis, orthodontic relapse.

p-ISSN: 1979-0201; e-ISSN: 2549-6212; Available from: <http://jurnal.unpad.ac.id/pjd/article/view/19848>

DOI: [10.24198/pjd.vol32no1.19848](https://doi.org/10.24198/pjd.vol32no1.19848)

Submission: Dec 21, 2018; Accepted: Mar 26, 2020; Published online: Mar 31, 2020

INTRODUCTION

Orthodontic treatment is aimed to obtaining a pleasant dentofacial appearance by removing

the malocclusion of the teeth, correcting the rotational and apical abrasion of the teeth, and correcting the incisive relation and creating a good occlusion relationship in order to improve

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individual's psychosocial health.^{1,2} Nowadays, needs for orthodontic treatment are significantly increase. Graber and Vanarsdall in Bhattarai said that adolescents who get orthodontic treatment in the United States have increased from 3% to 25% within 20 years.³ Aesthetic aspect is something that is very important for everyone and the one that influences it is the tooth appearance. A poor structure is called malocclusion, a condition of disharmony and between dental and arch that causes disruption in the balance of the function and mastication, speech, and social interaction.^{4,5,6} Orthodontic tooth movement is a combination of resorption and bone apposition on the side of pressure and tension.⁷ In the orthodontic treatment, there are two theories of tooth movement. The first theory is with the presence of collagen fibers from periodontal ligaments that connect teeth with alveolar bone. At the pressure area, the fibers will disappear causing the alveolar bone to be reduced, which is called resorption. The second theory is fluid redistribution in the periodontal ligament which causes rapid return of normal tissue fluid pressure in the periodontal space causing the apposition.⁸

But apparently, there is a problem that arises after the release of orthodontic device is the occurrence of relapse. The problem of relapse that faced by community is not small. One study showed 50% relapse seen in 2 years post retainer use, 28% relapse at 2-5 years after make retainer and 12% relapse seen at 5-10 years after make retainer. The results of the data processing show that the prevalence of relapse after orthodontic treatment is 70.83%.⁹

According to British Standard Institute, relapse is a condition in which the tooth structure returns to the initial form of malocclusion after the correction.¹⁰ To prevent relapse and to maintain changes in the dental tissue which are still developing, retention or retainer device are required.¹¹ Retainers are orthodontic passive device that play a role in stabilizing teeth for a long time to provide an opportunity to reorganize support structures after orthodontic treatment.⁴

The structure that affects of soft connective tissue or supporting the tooth consists of ligaments periodontal, processus alveolaris, cementum, and gingiva.^{12,13} Periodontal ligament is a soft connective tissue between the tooth root and

the inner wall of the alveolar socket. Periodontal ligament in contact with the teeth with alveolar bone which provides protection and support in the mastication system.¹⁴

Ligament periodontal as support of the movements have tooth that contains collagen, was formed from fibroblast and functions in the teeth through processus alveolaris.¹² Remodeling of the periodontal ligament plays an important role in the orthodontic field. The combination of periodontal ligament remodeling and the presence of apposition and resorption of alveolar bone can cause tooth to move.⁸ The cells are involved in remodeling takes the ligament periodontal is fibroblast, osteoblast, cementoblast, the vascular cells, cells hematopoietic the important as the recipient of the stress of a mechanical.¹⁵ Fibroblast a role in remodeling takes the bone and revitalization of the fibers of the ligaments periodontal of supporting the teeth to the stability of the treatment orthodontia. It takes 4 - 6 months tearing the cruciate ligament periodontal and the alveolar bone to support the teeth. If the orthodontic done before this phase, then there is relapse.¹⁶

Hyperbaric Oxygen Therapy (HBOT) is a therapy that uses 100% oxygen at a higher pressure than atmospheric pressure. The patient will inhale 100% oxygen gradually with increasing pressure in the therapy chamber into more than 1 absolute atmosphere (ATA).^{17,18} According to study of Ramadhani et al. HBOT 2,4 ATA was administered for 30x3 minute with a 5 minute interval for 7 days very useful in the orthodontic movement because it can stimulate the growth of new blood vessels thus stimulates the remodeling process leading to increased osteoblast activity.⁷

HBOT has benefits in the process of callus formation in bone remodeling and maintaining angiogenesis in remodeling processes.¹⁸ Based on Study of Sunnu, HBOT administration can accelerate the process of remodeling the periodontal ligament and alveolar bone in the tension area.¹⁹

Propolis is a natural substance made from honeybees, which act as antioxidant, antimicrobial and anti-inflammatory.

In general, propolis contains 50% resin and vegetable balsam, 30% wax, 10% aromatic and essential oils, 5% pollen and 5% other content,

including organic debris.²⁰ In 2011, Guney et al in Sartika et al examined the antioxidant effects of propolis in the healing process of femoral fracture in mice. The result of study showed that bone mineral density was higher.²¹ In propolis, there were antimicrobial agents of polyphenols that could function as anti-inflammatory.²² It was found that 3% of propolis content could be used to maintain oral hygiene in patient with cleft lip and propolis 5% in the mouthwash.²³

Based on previous literature and research studies on HBOT and propolis, and looking at limited studies on this combination that have an advantage to accelerate the proliferation of fibroblast in the remodeling of the ligament periodontal, the authors were interested in investigating and assessing the effect of HBOT 2.4 ATA for 3x30 minutes daily in 7 days and propolis extracts to knowing the amount of fibroblast and a width of the ligament periodontal to prevent relapse after orthodontic movement. Aim of this research is to analyze the difference of the width of the periodontal ligament and amount fibroblast in the tension area with combination of propolis gel and HBOT in an attempt to prevent occurrence of relapse.

METHODS

This study was an experimental laboratory. The samples that used in this study was guinea pigs with selected criteria were male sex, age 2-3 months, weight 350-550 grams. Ethical clearance of this research was obtained from the Ethics and Scientific Research Committee of Experimental Animal Use in the Faculty of Dentistry Hang Tuah University Surabaya (No : 048/KEPK/I/2018). The male guinea pigs (n=42) divided into 7 groups i.e. untreated groups ((K(-)), group who only given rubber separator ((K (+)), a group who given 3% propolis gel (P1), a group who given 5% propolis gel (P2), a group who given Hyperbaric Oxygen Therapy 2.4 ATA (P3), a group who was given 3% propolis gel combination and HBOT (P4), and the group who was given a combination of HBOT 2.4 ATA and 5% propolis gel.

Propolis gel was applied in gingiva sulcus with insulin syringe 0,025 ml per day. Hyperbaric oxygen therapy was conducted by using an animal chamber, with 100 % pure oxygen at 2.4 ATA for 90

minutes in 7 days. The Guinea pig were euthanasia after day 16 and maxilla was coronal plane and performed, Afterwards, histological section was prepared with HE staining and then observed by using microscope. The photo were taken to measure the number of fibroblast seen on the microscope with an enlargement 400x.

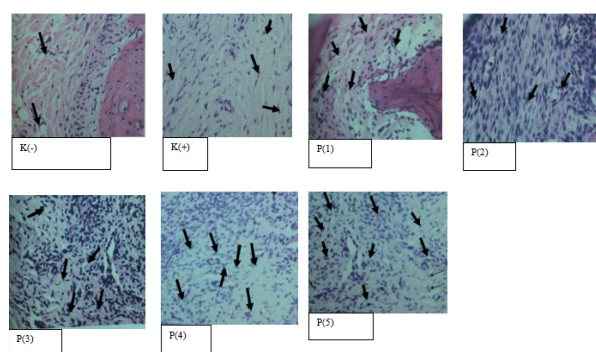


Figure 1. Histology of fibroblast in the tension area with HE staining and 400 x magnification

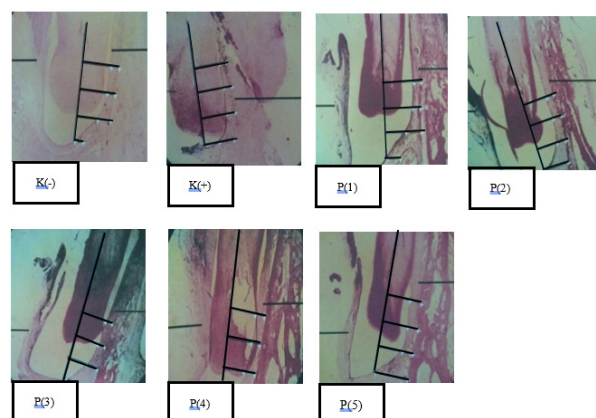


Figure 2. Histology of ligament periodontal in tension area with HE staining and 100 x magnification

Table 1. Results of the average the amount of fibroblast

Group	Mean \pm Standard Deviation
K(-)	29.17 \pm 1.169
K(+)	42.83 \pm 2.927
P1	52.33 \pm 1.211
P2	63.33 \pm 2.338
P3	76.50 \pm 2.258
P4	85.50 \pm 1.761
P5	95.67 \pm 2.422

Meanwhile, the size of the periodontal ligament on 1/3 apical in the tension site was observed with enlargement 40x. Each histological section was observed and calculated as many as three times in the field of view. The data were statistically measured by using Statistical Package

Table 2. Results of the average width of the periodontal ligament

Group	Mean ± Standard Deviation
K(-)	0.52±0.07
K(+)	0.63±0.11
P1	0.75±0.09
P2	0.79±0.03
P3	0.97±0.16
P4	1.01±0.14
P5	1.03±0.15

for the Social Science (SPSS) version 20. The statistically significant differences among group were determined and evaluated by LSD test ($p < 0.05$).

RESULTS

Data from fibroblast in the area from the calculation of the fibroblast in the figure of the

Table 3 Results of post-hoc LSD test of fibroblast

Rata Kel	K(-)	K(+)	P1	P2	P3	P4	P5
K(-)		0,000*	0,000*	0,000*	0,000*	0,000*	0,000*
K(+)			0,000*	0,000*	0,000*	0,000*	0,000*
P1				0,000*	0,000*	0,000*	0,000*
P2					0,000*	0,000*	0,000*
P3						0,000*	0,000*
P4							0,000*
P5							

Description: * significant at $\alpha = 0.05$

histology in all the groups. Histology the amount of fibroblast in the K (-), K (+), P1, P2, P3, P-4, and P5 as in the figure 1.

Based on the average amount of fibroblast on orthodontic tooth movement in an effort to prevent relapse, the average value of the amount

Table 4 Results of post-hoc LSD test of periodontal ligament

Rata Kel	K(-)	K(+)	P1	P2	P3	P4	P5
K(-)	0.52	0.63	0.75	0.79	0.97	1.01	1.03
K(+)	0.094						
P1	0.001*	0.081					
P2	0.000*	0.020*	0.528				
P3	0.000*	0.000*	0.002*	0.012*			
P4	0.000*	0.000*	0.000*	0.002*	0.527		
P5	0.000*	0.000*	0.000*	0.001*	0.386	0.813	

Description: * significant at $\alpha = 0.05$

of fibroblast highest is group P5 (95.67) and the average value of the amount of fibroblast is a lowest group of K- (29.17).

Based on the average width of the periodontal ligament table on orthodontic tooth movement in an effort to prevent relapse, the average value of the width of the periodontal ligament highest P5 group (1:03) and the average value of the width of the periodontal ligament is a group of K- lowest (0.52). This means giving the combination of HBOT 2.4 ATA and 5% propolis gel most effective in preventing relapse.

Post-Hoc LSD test results showed significant differences in group K (-) with group K(+), P(1), P(2), P(3), P(4) and P(5); Group K(+) with P(1), P(2), P(3), P(4), and P(5); Group P(1) with group P(2), P(3), P(4), and P(5); Group P(3) with group P(4) and P(5); and between group P(4) and group P(5).

DISCUSSION

This research was aimed to investigate the amount of fibroblast and the difference of width of periodontal ligament in the untreated tension site, 3% Propolis therapy, 5% Propolis, Hyperbaric Oxygen Therapy (HBOT), a combination of HBOT with Propolis 3%, and HBOT combination with 5% Propolis in an effort to prevent relapse. Animals used in this study were guinea pigs (*Cavia cobaya*) because it is easy to control and very appropriate to study the orthodontic tooth movement. The histology of guinea pigs teeth counts more easily than other animals.²⁴

Orthodontic treatment is based on the principle that when a pressure is applied to the teeth with a certain time period, there will be a movement of teeth due to periodontal ligament and bone around the teeth changes (remodelling). This orthodontic forces will cause tissue trauma, periodontal ligament compression, and bone deformation. This incident is followed by a biochemical reaction at the cellular level, which results in bone remodelling.²⁵ The orthodontic forces in the tooth produce a pressure and tension site. Pressure area is an area periodontium under pressure because the teeth move closer and tension site is the area periodontium been pulled because the teeth move away.

The pressure area will undergo bone resorption while the tension area will experience

bone deposition.²⁶ Compare to this study, on days 1 and 2 a 0.028 kN separator rubber was used so those male guinea pigs could adapt to the mechanical strength of the separator rubber, reduce the pain, and obtain small tooth movement. Then on the third day the replacement of the rubber separator with the strength of 0.474 kN and larger size.

Analysis of this separator changed after day 1 because there are small tooth movements and to avoid the loss of rubber separator with smaller strength and size. Then there was remodelling on the alveolar bone so that eventually there was a movement of teeth. Periodontal ligaments have an important role in the movement of teeth in the orthodontic process. In order for the tooth to move in the periodontal ligament, osteoclasts must form an osteoclast that resembles the bone adjacent to the depressed periodontal ligament. While on the tension area formed by osteoblast progenitor cells in the periodontal ligament subsequently form new bone. The strength of an orthodontic device in this study using a rubber separator, which will be responded by the periodontal ligament.²¹

Orthodontic devices as mechanical stimuli in the early stages of child care make a biological cellular response to the periodontal ligament in the form of inflammation, followed by the release of T-cell cytokines, B-cells and matrix metalloproteinase (MMP) enzymes.²⁷ In periodontal tissue remodelling, there are many metabolic processes involve fibroblast cells, osteoblasts, cementoblasts, and vascular cells as recipients of mechanical stress.²⁸ When orthodontic pressure is applied to the teeth, the cells in the periodontal ligament will respond to these mechanical forces that leads to the alveolar bone remodeling thus moving the teeth.¹¹

All elements in the human body will return to their normal state. Similarly, the condition of orthodontic strained ligaments can returned to its original state if it is not given retention, which is often called a relapse. Relapse is a tendency to return to its original position as before orthodontic treatment and generally more common to be occurred in the lower anterior teeth.²⁹ Dianastesi stated that the prevalence of relapse after fixed orthodontic treatment was 70.83%.²⁹ Adjuvant therapy with propolis and HBOT is expected to help prevent relapse.⁹

Giving hyperbaric oxygen can lead to proliferation of fibroblasts, collagen synthesis, increased leukocytes, and angiogenesis that causes neovascularization in the pull region.^{30,31} The formation of new blood vessels (neovascularization) thus stimulating the remodelling process and some growth factors, such as prostaglandin (PG), vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF).

The factors HBOT administration has the power and influence that support the recovery process regeneration of tissues. High- pressure oxygen exposure results in increased IFN- γ , I-NOS and VEGF, IFN- γ causing TH-1 binding that affects B-cells resulting in increased Ig-G.³² By giving a pressure of 2.4ATA, then the partial arterial pressure will increase 10 times so that the concentration of oxygen in the blood flow will increase by ten times the normal level.³³ The blood flow in the periodontal ligament will affect the thickness. In depressed periodontal ligaments, blood flow and oxygen supply decrease, whereas, in interested periodontal ligaments, the blood supply remains or increases. With this mechanism, it can be said that the hyperbaric oxygen can increase oxygen concentration, neovascularization, angiogenesis in remodeling.

Proved that by giving hyperbaric oxygen 2.4 ATA, 90 minutes, for 7 days there was an increase of osteoblast cell number in male guinea pigs.³⁴ Compare with these studies show that hyperbaric oxygen delivery stimulates the formation of new blood vessels that can stimulate the number of fibroblast and increased the width of periodontal ligament remodelling process during the movement of teeth.

Propolis contains anti-inflammatory and antimicrobial ingredients. Propolis not only serves to decrease apoptosis but also can increase metabolic activity and proliferation of periodontal ligament cells.³⁵ The highest content of propolis is flavonoids that can stimulate the development of basic Fibroblast Growth Factor (bFGF) and TGF- β 1 so as to increase collagen synthesis.³⁶

Propolis contains various variations of fatty acids such as the 10HDA molecular structure. These fatty acids increase the production of fibroblasts by stimulating TGF- β 1 to produce fibroblasts and collagen.³⁷ There is two glycoprotein content

in propolis, namely vitronectin, and laminin, which can increase the amount or proliferation of fibroblasts by stimulating TGF- β expression, in which TGF- β induces bFGF-forming fibroblast cells.³⁸

Based on previous research, it was found that propolis gel given in 14 days can reduce inflammation and increase fibroblasts, so we conduct our research by giving Propolis extract in gingival sulcus for 14 days. It also found from previous study that 3% propolis content can be used to maintain oral hygiene in patients with cleft lip and 5% propolis in mouthwash.²⁵ If fibroblasts can be precisely induced, they may form periodontal ligaments.³⁹

In this study we conduct to removed the separator rubber after 14 days giving the mechanical forces, then removed the separator for 2 days, there will be a relapse. The main cause of relapse is that the gingival and periodontal tissues affected by orthodontic tooth movement require reorganization time when the appliance is removed even after the retention device is installed.⁴ Based on the results of the study, after the mechanical strength of orthodontic removed, relapse occurs and the position of the teeth begins to move back.

In the table, P (1) shows that group which given orthodontic mechanical pressure for 14 days and released on days 15-16 and given additional therapy propolis 3% on days 3-16 had an increase in amount of fibroblasts and width of ligaments in the tension area in the relapse process. According to the statistical results, the number of fibroblasts in tension area is increased and the width of the ligaments in the tension area is wider than the group given only the mechanical strength of orthodontics without any treatment. This is happen because by giving Propolis with content of flavonoids that have antibacterial properties, affect the permeability of blood vessels and blood flow, affect the cardiovascular system.

Flavonoids in propolis are also anti inflammatory and have the potential to stimulate the formation of collagen in order to increase bone, tissue and cartilage regeneration. Propolis contains a wide variety of fatty acids 10HDA case of the molecular structure. These fatty acids increase the production of fibroblasts by stimulating TGF- β 1 to produce fibroblasts and

collagen.³⁸ The result showed that the difference of ligaments with the untreated group, where the group given propolis 3% had a wider ligament width. However, based on the descriptive test results showed no significant difference with the group who did not receive therapy. Then propolis 3% less effective in preventing relapse.

In the table, P (2) shows that group which given orthodontic mechanical pressure for 14 days and released on days 15-16 and given additional therapy 5% propolis on days 3-16 had an increase in ligament width and amount of fibroblasts in the tension area in the relapse process. Compare to Pieniazek.

found that 3% of propolis content could be used to maintain oral hygiene in patient with cleft lip and propolis 5% in the mouthwash, the result of this study also giving best result. Propolis contains Caffeic Acid Penythyl Ester (CAPE) and carbohydrates in the form of pentoses and monosaccharides that can synthesize fibroblasts and collagen.³⁹ This is done by stimulating the development of bFGF and TGF- β . Cinnamic acid and ascorbic acid in propolis play a role in the proliferation of fibroblasts and the formation of collagen fibers.

Based on the results of a descriptive test of the study found a significant difference in the width of the periodontal ligament and amount fibroblast in the tension site in the group given 5% propolis with the untreated group. However, there was no significant difference with the group given propolis 3%. So propolis 5% more effective in preventing the relapse process compared with propolis 3%, because of the content of flavonoids in propolis 5% greater than the propolis 3%.

In the table, P (3) group which given orthodontic mechanical pressure for 14 days and released on days 15-16 and were given HBOT supplementary therapy on days 10-16 there was an increase in amount of fibroblasts in the width of the periodontal ligaments in the tension area in the relapse process HBOT increases oxygen in the bloodstream and increases ROS (Reactive Oxygen Species), which acts as a signal molecule in the development of several growth factors, namely basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), and transforming growth factor (TGF- β 1), which can increase the activity of the cells for angiogenesis.

Angiogenesis plays an important role in remodelling by increasing the osteoblast activity that can synthesize and secrete collagen for remodelling of periodontal ligaments.^{7,18,19} Through the krebs cycle there is an increase in hydrogen adenosine nicotinamide dinucleotide (NADH) which triggers an increase in fibroblast.

Fibroblast proliferation is affected by oxygen concentration.³² Based on the results of the descriptive test of the study found a significant difference between the groups given HBOT for 7 days compared with the group not given HBOT. This supports the theory that HBOT increase the periodontal ligament remodelling.

In the table, P (4) group which given orthodontic mechanical pressure for 14 days and released on days 15-16 and given a combination of 3% propolis gel on days 16-16 and HBOT on days 10-16 there was an increase in amount of periodontal ligamentous wide fibroblasts in the tension area in the relapse process. At the time of administration of HBOT, the concentration of oxygen in the bloodstream rises so that all body fluids running very quickly so that oxygen can reach the bone and soft tissue damaged that cannot be penetrated by the red blood cells and can enhance the function of white blood cells, which increases the process of neovascularization and vascular resulting in rapid healing.⁴⁴ Propolis can decrease apoptosis and increase metabolic activity and proliferation of periodontal ligament cells.³⁹

Based on the results of statistical tests it was found that the amount of fibroblast and width of the ligaments was greater in the group given 3% propolis gel and HBOT compared to those given the HBOT only. It's happen because sinergical effect between HBO who administrated systemically to the body and Propolis extract that given topically. In the table, P (5) group given orthodontic mechanical pressure for 14 days and released on days 15-16 and given a combination of 5% propolis gel on days 3-16 and HBOT on days 10-16 experienced an increase in periodontal ligament width and amount fibroblasts in the tension area in the relapse process.

According to the statistical result, in the group given the combination gel, propolis 5% and HBOT has a amount of fibroblast and width of the periodontal ligament of the greatest compared to other treatment groups. This is because it has

been demonstrated that systemic HBOT therapy improves the remodelling of periodontal ligaments and 5% propolis therapy locally contains fatty acids that can increase fibroblast cells which are the main cells in the periodontal ligament.

However, the difference between the width of the periodontal ligament HBOT group was only given to the group given combination therapy propolis gel 3% and HBOT with the group given combination therapy gel propolis 5% and HBOT is not significant. This proves that HBOT is the most important therapy in this study compared with 3% propolis gel and 5% propolis gel.

CONCLUSION

The combination of HBOT and propolis gel affect the width of periodontal ligament and the amount of fibroblast in the area of the realps orthodontic.

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