

# Dental Journal

Majalah Kedokteran Gigi



The increased number of osteoblasts and capillaries in orthodontic tooth movement postadministration of Robusta coffee extract (*coffea canephora*)  
· The influence of artificial salivary pH on nickel ion release and the surface morphology of stainless steel bracket-nickel-titanium archwire combinations · Increase of collagen in diabetes-related traumatic ulcers after the application of liquid coconut shell smoke



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### Research Report

## The increased number of osteoblasts and capillaries in orthodontic tooth movement post-administration of Robusta coffee extract

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### ABSTRACT

**Background:** The application of orthodontic forces subjects blood capillaries to considerable pressure, resulting in hypoxia on the pressure side. Vascular endothelial growth factor (VEGF), expressed in osteoblasts represents an important mitogen that induces angiogenesis. Osteoblasts and blood capillaries play an important role in bone formation. Robusta coffee contains chlorogenic acid and caffeic acid both of which produce antioxidant effects capable of reducing oxidative stress in osteoblasts. **Purpose:** The aim of this study was to analyze the effects of Robusta coffee extract on the number of osteoblasts and blood capillaries in orthodontic tooth movement. **Methods:** This research constituted a laboratory-based experimental study involving the use of sixteen male rodents divided into two groups, namely; control group (C) consisting of eight mice given orthodontic mechanical force (OMF) and a treatment group (T) containing eight mice administered OMF and dried Robusta coffee extract at a dose of 20mg/100 g BW. The OMF was performed by installing a ligature wire on the maxillary right first molar and both maxillary incisors. In the following stage, the maxillary right first molar was moved to the mesial using Tension Gauze with a Nickel Titanium Orthodontic closed coil spring. Observation was subsequently undertaken on the 15th day by extracting the maxillary right first and second molar with their periodontal tissues. Thereafter, histological examination was performed using hematoxylin-eosin (HE) staining technique to measure the number of osteoblasts and blood capillaries on the mesial and distal periodontal ligaments of the maxillary right first molar. **Results:** The administration of Robusta coffee extract increases the number of blood capillaries and osteoblasts on both the pressure and tension sides were found to be significantly higher in the T group compared to the C group ( $p < 0,05$ ). **Conclusion:** Robusta coffee extract increase the number of osteoblasts and blood capillaries, thereby playing a role in improving the alveolar bone remodeling process in orthodontic tooth movement.

**Keywords:** orthodontic tooth movement; Robusta coffee; VEGF; capillary; osteoblasts

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### INTRODUCTION

Orthodontic tooth movement depends on the remodeling of periodontal ligament and alveolar bone associated with a number of biological and mechanical responses of the surrounding tissue. Subjecting periodontal ligaments to pressure will result in bone resorption, whereas placing periodontal ligaments under tension will lead to bone formation.<sup>1</sup> In other words, bone resorption is effected by osteoclasts on the pressure side, while a new bone formation is produced by osteoblasts on the tension side.<sup>2</sup>

Osteoblasts are bone-forming cells that express parathyroid hormone receptors and execute several important roles in bone remodeling, namely; osteoclastogenic factor expression, bone matrix protein production, and bone mineralization.<sup>3</sup> At the tension site, new bone is formed as a result of orthodontic force during treatment.<sup>4</sup> Osteoblast cells are involved in bone formation which commences some 40-48 hours after the initial application of orthodontic force. Differentiation of osteoblasts subsequently begins with stem cells derived from bone marrow moving to the blood vessels. The migration of mesenchymal stem cells



from the blood vessel wall or the activation of mesenchymal stem cell precursors and preosteoblasts formation occurs approximately 10 hours after the application of orthodontic force.<sup>5</sup> Mature osteoblasts form osteoid leading to a mineralization process,<sup>4</sup> with endothelial nitric oxide synthase (eNOS) then facilitating bone formation at the tension site.<sup>6</sup>

Furthermore, periodontal ligaments consist of blood capillaries, very small blood vessels, at the end of the arteries. The walls of blood capillaries consist of a thin endothelial layer and lymph fluid that can be secreted to form a tissue fluid capable of carrying nutrients, including water and minerals, to cells. Through a process of gas exchange between the capillary blood vessels and cells in the tissues, the former can both provide oxygen and remove carbon dioxide. In this manner, blood capillaries play an important role in distributing to the tissues important substances used for various processes in the body.<sup>7</sup>

The application of orthodontic force can narrow the blood vessels, thereby reducing the blood supply and causing hypoxia at the pressure site. Hypoxia constitutes a deficiency of oxygen in the tissues due to decreased partial oxygen pressure beyond the physiological level.<sup>8</sup> Hypoxia can also compromise cellular energy levels by reducing glycolytic activity and adenosine tri phosphate (ATP) production. Cells then respond to hypoxia by expressing cellular mediators, especially hypoxia-inducible factor 1 (HIF-1) that can promote angiogenesis, stimulate cell proliferation, and prevent cell death.<sup>9</sup>

Moreover, hypoxia induces the formation of an active transcription factor HIF-1 and activates genes that encode endothelial vascular growth factor (VEGF)<sup>9</sup> known as one of the most important mitogens to induce angiogenesis.<sup>10</sup> Angiogenesis is the growth of new blood vessels from existing ones. By binding to endothelial cell receptors, VEGF activates a signal cascade, resulting in various cellular and blood vessel reactions.<sup>11</sup>

When the VEGF binds to its receptor a signal is generated on the surface of the activated endothelial cell which is then sent to the cell nucleus. New molecules are produced by endothelial cell organelles, namely; protease enzymes, which serve to destroy the extracellular matrix as a branching point for capillary vessels. Angiopoietin growth factors and a matrix metalloproteinase enzyme produced by endothelial cells are required to initiate the formation of new blood vessels.<sup>12</sup> Matrix metalloproteinase (MMP) is also important for angiogenesis because of its role in extracellular matrix degradation, resulting in the migration of endothelial cells.<sup>9</sup> MMP-2 is even considered a direct transcriptional target of HIF-1 $\alpha$ , mediating endothelial cell migration in response to hypoxia.<sup>13</sup> In general, VEGF expression is detected in osteoblasts, osteocytes, and fibroblasts at the tension site after 10 days of orthodontic tooth movement.<sup>14</sup>

Unfortunately, relatively protracted orthodontic treatment constitutes a major problem for patients since it is often associated with a variety of conditions such as

dental caries,<sup>15</sup> external root resorption,<sup>16</sup> and open gingival embrasures.<sup>17</sup> Therefore, orthodontic tooth movement needs to be accelerated in several ways, one of which is through the use of additional medicines.<sup>18</sup>

Crucially, coffee represents one of the popular beverages consumed by societies.<sup>19</sup> Robusta coffee contains chlorogenic acid, phenylalanines formed during the roasting process,<sup>20</sup> and caffeic acid, which produce antioxidant effects in reducing oxidative stress in osteoblasts<sup>21</sup> and stimulate osteoblast activity.<sup>22</sup> Moreover, chlorogenic and caffeic acids in Robusta coffee produce antioxidant effects in enhancing angiogenesis through increased VEGF.<sup>23</sup> Results of research on mice even showed that the administration of Robusta coffee at a dose of 20 mg/100 g BM (equivalent to one cup of coffee in humans) increased the number of osteoclasts on the 15th day.<sup>24</sup> The study reported here aimed to analyze the increased number of osteoblasts and blood capillaries in orthodontic tooth movement after the administration of Robusta coffee extract

## MATERIALS AND METHOD

This research was a laboratory-based experimental study conducted over a period of three months at the Department of Biomedicine, Faculty of Dentistry, Universitas Jember. This research was approved by the Ethics Commission of the Faculty of Dental Medicine, Universitas Airlangga, Surabaya number: 8/KKEPK.FKG/II/2015.

This research involved sixteen healthy, male mice (Sprague Dawley) weighing 250-300 grams and aged between 3 and 4 months. They were divided into two groups, namely a control group (C) consisting of eight mice who were given orthodontic mechanical force (OMF) and a treatment group (T) consisted of eight rats given OMF and freeze-dried Robusta coffee extract with a dose of 20 mg/100 g BW (equivalent to one cup of coffee in humans) dissolved in 2ml of aquades. Coffee extract was given orally via a stomach sonde over a period of 14 consecutive days.

Subsequent to this, the mice were anesthetized with ketamine before an OMF was applied. A ligature wire (3M Unitek, Germany) 0.20 mm in diameter was mounted by attaching it to the maxillary right first molar and both maxillary incisors. Afterwards, the maxillary right first molar was moved to mesial using a tension gauge (Ormco, USA) to generate 10 g/cm<sup>2</sup> strength with a nickel titanium orthodontic closed coil spring (3M Unitek, Germany) 6 mm in length. Therefore, this closed coil spring extending from the maxillary right first molar to both maxillary incisors.<sup>25</sup> Observation was conducted by means of the rats being sacrificed on day 15 before their maxillary right first and second molar were extracted together with periodontal tissue. Histological examination was then performed using hematoxylin-eosin (HE) staining technique to observe the number of osteoblasts and blood capillaries on the mesial and distal areas of periodontal ligaments. Observations were



conducted using a microscope at 400x magnification. The mesial area represented the pressure side, while the distal area represented the tension side of the maxillary right first molar. The data obtained was subsequently analyzed by means of an Independent t-test, Mann Whitney test, Paired t-test and Wilcoxon signed ranks test with a confidence level of 95% ( $\alpha = 0.05$ ).

## RESULTS

The results of this research showed the mean  $\pm$  standard deviation of osteoblasts at the pressure sides in C group and T group to be  $2.88 \pm 0.99$  and  $7.50 \pm 1.69$ , respectively; whereas at the tension side it was  $4.13 \pm 1.64$  and  $11.50 \pm 1.20$ , respectively. This confirmed that the number of osteoblasts in both the pressure and tension sides in group T was greater than those in Group C. These results strongly suggest that the number of osteoblasts at the tension side was greater than at the pressure side in both of Group C and T. In addition, it suggests that the number of osteoblasts at the tension side was greater than at the pressure side of both research groups.

At this point, a difference test was performed on the number of osteoblasts at the pressure side of Groups C and T using a Mann-Whitney test since the data was not normally distributed ( $p < 0.05$ ). The results showed that the number of osteoblasts in the pressure sides was found significantly higher in the T group compared to the C group ( $p < 0.05$ ). On the other hand, an Independent t-test was conducted on the number of osteoblasts at the tension side of the C and T groups since the data was normally distributed ( $p > 0.05$ ). The results of the Independent t-test indicated that the number of osteoblasts in the tension sides in the T group was found to be significantly higher than that of the C group ( $p < 0.05$ ). A Wilcoxon signed rank test was then conducted to reveal the difference in osteoblasts between the pressure and the tension sides of both research groups since the data were not normally distributed ( $p < 0.05$ ). The results indicated that the number of osteoblasts in the C group was not found to be significantly higher in the tension side compared to the pressure side ( $p > 0.05$ ), while in the T

group there was a significantly higher in the tension side compared to the pressure side ( $p < 0.05$ ).

The results also showed that the mean  $\pm$  standard deviation of blood capillaries at the pressure sides in C and T groups was  $3.20 \pm 0.11$  and  $4.30 \pm 0.38$ , respectively; while on the tension sides it was  $3.31 \pm 0.13$  and  $5.14 \pm 0.21$ , respectively. This indicated that the number of blood capillaries on both the pressure and the tension sides in T group was greater than in C group. Furthermore, it suggests that the number of blood capillaries at the tension side was greater than at the pressure side in both research groups.

An independent t-test was subsequently performed on the number of blood capillaries in both the pressure and the tension sides of C group and T group since the data were normally distributed ( $p > 0.05$ ). The results showed that the number of blood capillaries in both the pressure and the tension sides was found significantly higher in the T group compared to the C group ( $p < 0.05$ ). Paired t-test then was conducted to reveal the difference of blood capillaries between the pressure and the tension sides of both research groups since the data were normally distributed ( $p > 0.05$ ). The paired t-test results indicated that the number of blood capillaries in the C group was not significantly higher in the tension side compared to the pressure side ( $p > 0.05$ ), while in T group it was significantly higher in the tension side compared to the pressure side ( $p < 0.05$ ) (Table 1).

A histological examination was carried out on the number of osteoblasts and blood capillaries in the periodontal ligaments. The results indicated that the number of osteoblasts and blood capillaries on both the pressure and the tension sides was higher in the mice treated with the Robusta coffee extract than in the control group (Figure 1). Figure 1 illustrates the number of osteoblasts and capillaries on the pressure side in C group not treated with the Robusta coffee extract (Figure 1-A) and in T group treated with the Robusta coffee extract (Figure 1-B). In addition, the number of osteoblasts and blood capillaries in the tension side of C group not treated with the Robusta coffee extract can be seen in Figure 1-C. Meanwhile, Figure 1-D demonstrated the number of osteoblasts and blood capillaries in the tension side of T group treated with the Robusta coffee extract.

**Table 1.** The mean  $\pm$  standard deviation of osteoblasts and blood capillaries at the pressure and tension sides

Group	n	Osteoblasts (Mean $\pm$ Standard deviation)			Blood capillaries (Mean $\pm$ Standard deviation)		
		Pressure	Tension	p	Pressure	Tension	p
C	8	$2.88 \pm 0.99$	$4.13 \pm 1.64$	0.156	$3.20 \pm 0.11$	$3.31 \pm 0.13$	0.135
T	8	$7.50 \pm 1.69$	$11.50 \pm 1.20$	0.012	$4.30 \pm 0.38$	$5.14 \pm 0.21$	0.007
p		0.000	0.000		0.000	0.000	

Note:  $p < 0.05$  = Significant difference;  $p > 0.05$  = no significant difference



## DISCUSSION

Orthodontic force can decrease blood flow on the pressure side as the blood vessels are depressed while, conversely, blood flow on the tension side increases. Such changes in the blood flow will alter the blood chemistry.<sup>26</sup> The reduced oxygen supply in the pressure zone will trigger a hypoxic condition in the periodontal capillaries. Local hypoxia will then increase VEGF expression in fibroblasts of the periodontal ligament, while also potentially inducing the formation of an active HIF-1 transcription factor and activating genes encoding VEGF.<sup>9</sup> Consequently, orthodontic tooth movement can accelerate osteoblast activity, resulting in increased VEGF expression.<sup>27,28</sup>

The results of this research showed that the administration of Robusta coffee extract enhanced the number of osteoblasts at the pressure and tension sides of the T group compared to those of the untreated C group. This was because the antioxidant effects of chlorogenic acid and caffeic acid contained in the coffee extract can reduce oxidative stress in osteoblasts, thereby increasing the differentiation and stability of osteoblasts.<sup>21,28</sup>

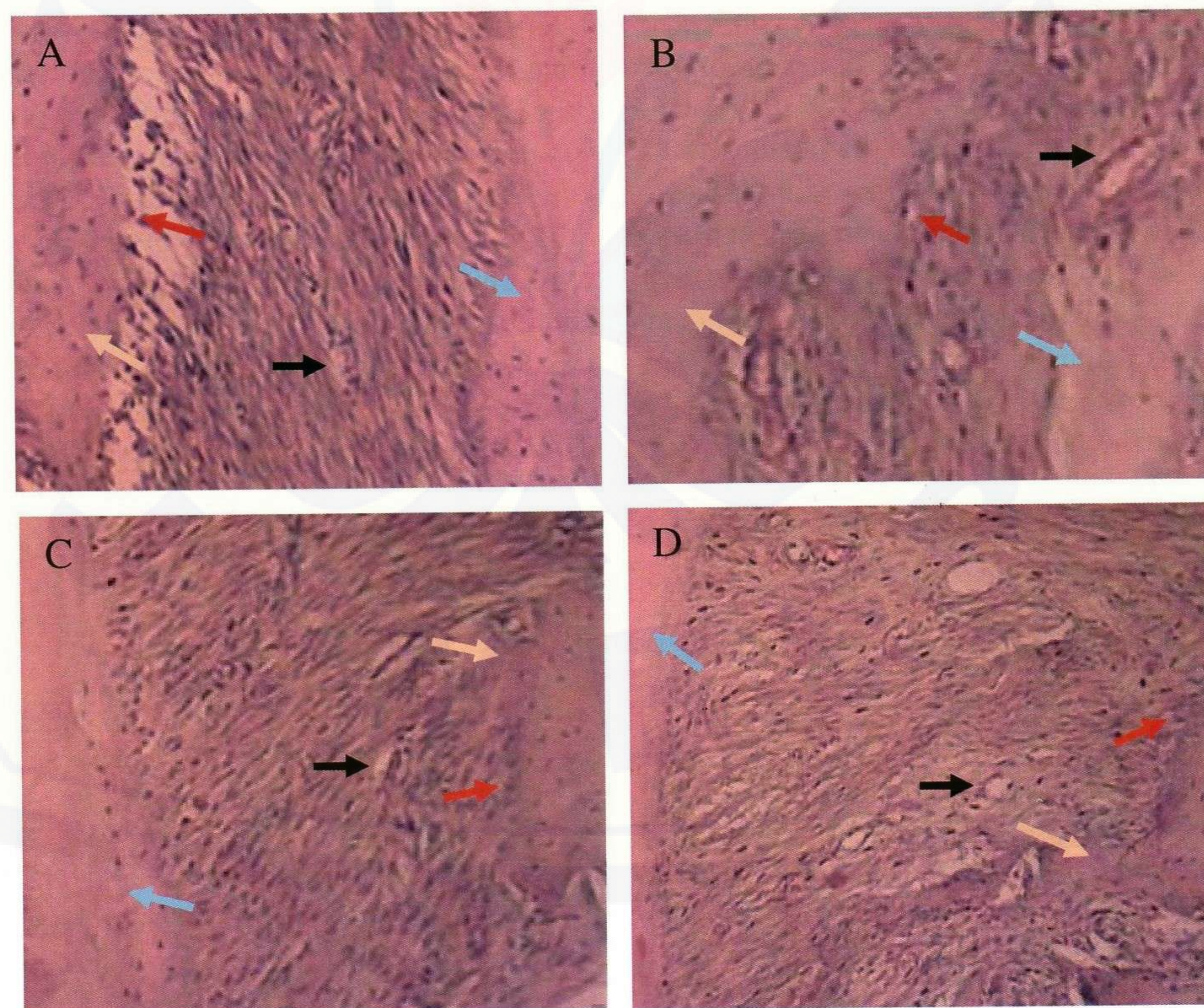
Orthodontic tooth movement can also trigger the occurrence of reactive oxygen species (ROS) which can then increase lipid peroxidation, the main cause of damage to the cell membranes, disrupting the structure and function of osteoblast cells. Thus, chlorogenic acid and caffeic

acid contained in the coffee extract will act as antioxidant agents to protect cells by converting free radicals into stable products, thereby preventing osteoblast cell membrane damage.<sup>28</sup>

The results of the research also indicated that the Robusta coffee extract increased the number of blood capillaries at both the pressure and tension sides of T group compared to the C group that was not treated with the Robusta coffee extract. This could be caused by osteoblasts expressing VEGF which stimulated angiogenesis.<sup>10</sup> In other words, the increased number and activity of osteoblasts due to the administration of Robusta coffee extract can generate VEGF expression.<sup>27,28</sup> Angiogenesis enhances the number of blood capillaries since the width of the major blood vessel plexus significantly increases because of branched capillaries which will then transform those branched capillaries into perfect vascular tissue.<sup>29</sup>

Angiogenesis begins with the degradation of the pre-existing blood vessel wall before activating the proliferation and migration of endothelial cells. The endothelial cells are arranged in a tubular structure around the walls of the blood vessels that will be formed. Endothelial end cells then stimulate shoots to grow, with the result that new blood vessels will be developed and the dilation and replenishment of blood containing oxygen will occur.<sup>30</sup>

In this research, the administration of Robusta coffee extract further increased the number of osteoblasts and blood



**Figure 1.** Osteoblasts (red arrows) and capillaries (black arrows) on the pressure side of the control group (A), the pressure side of the treatment group (B), the tension side of the control group (C), and the tension side of the treatment group (D). Alveolar bones (yellow arrows), while the roots of the tooth (blue arrows) (HE staining, 100x magnification).



capillaries at the tension side compared to the pressure side. This scenario could be caused by the antioxidant effects of the chlorogenic acid and caffeic acid contained in the coffee extract not only increasing osteoblast differentiation, but also promoting osteoblast activity.<sup>22,28</sup> Consequently, both VEGF expression and the number of capillaries increase.

The area of the tension side is larger than that of the pressure side with the result that a higher number of osteoblasts and blood capillaries is required in bone formation. This finding is consistent with the results of the study showing that Robusta coffee brewing increases the incidence of bone islands and VEGF in tension areas to a greater extent than in pressure areas.<sup>24</sup>

Blood vessels actually play a key role in the remodeling of bone growth and development.<sup>5</sup> The process of bone formation is related to the development of new capillaries within the existing blood vessels.<sup>31</sup> Osteoblasts playing an important role in bone formation are required to remodel the resorption region on the pressure side and to form new bone on the tension side.<sup>32</sup> Osteoblasts are differentiated from mesenchymal cell cursors, while mature osteoblasts form osteoids, followed by bone mineralization process.<sup>4</sup>

In addition, VEGF can modulate the recruitment, differentiation, and activation of osteoclast precursors, thereby increasing bone resorption. VEGF also indirectly leads to bone resorption since it promotes angiogenesis in vitro, enabling new capillaries to assist in the recruitment of osteoclasts to the nearest bone surface and then to the resorption area.<sup>13</sup> In vitro research has confirmed that VEGF can stimulate osteoclast differentiation by increasing the RANKL/OPG ratio. RANKL (a receptor activator of nuclear factor kb ligand) and OPG (Osteoprotegerin) are osteoblast-generated cytokines and VEGF plays an important role in the proliferation, migration, and invasion ability of osteoblasts.<sup>33</sup> Finally, the active osteoclasts will result in bone resorption, with osteoblasts therefore also indirectly playing a role in bone resorption.<sup>34,35</sup> In conclusion, Robusta coffee extract can increase the number of osteoblasts and blood capillaries that play an important role in orthodontic tooth movement.

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