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The Inhibition of Relapse of Orthodontic Tooth Movement by NaF Administration in Expressions of TGF-β1, Runx2, Alkaline Phosphatase and Microscopic Appearance of Woven Bone

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***Abstract*—**The prevalence of post-treatment relapse in orthodontics in the community is high enough; therefore, relapses in orthodontic treatment must be prevented well. The aim of this study is to experimentally test the inhibition of relapse of orthodontics tooth movement in NaF of expression TGF-β1, Runx2, alkaline phosphatase (ALP) and microscopic of woven bone. The research method used was experimental laboratory research involving 30 rats, which were divided into three groups. Group A: rats were not given orthodontic tooth movement and without NaF. Group B: rats were given orthodontic tooth movement and without 11.5 ppm by topical application. Group C: rats were given orthodontic tooth movement and 11.75 ppm by topical application. Orthodontic tooth movement was conducted by applying ligature wires of 0.02 mm in diameter on the molar-1 (M-1) of left permanent maxilla and left insisivus of maxilla. Immunohistochemical examination was conducted to calculate the number of osteoblast to determine TGF β1, Runx2, ALP and haematoxylin to determine woven bone on day 7 and day 14. Results: It was shown that administrations of Natrium Fluoride topical application proved effective to increase the expression of TGF-β1, Runx2, ALP and to increase woven bone in the tension area greater than administration without natrium fluoride topical application ($p < 0.05$), except the expression of ALP on day 7 and day 14 which was significant. The results of the study show that NaF significantly increases the expressions of TGF-β1, Runx2, ALP and woven bone. The expression of the variables enhanced on day 7 compared on that on day 14, except ALP. Thus, it can be said that the acceleration of woven bone occurs on day 7.

***Keywords*—**TGF-β1, Runx2, ALP, woven bone, natrium fluoride.

I. Introduction

THE purpose of orthodontic treatment for adjusting teeth in the arch teeth correctly, which improves the function of chewing, aesthetic, facial harmony, health, and mouth chain tooth position stability after treatment, as well as moving the teeth with minimum adverse effects on the teeth and periodontal tissue [1]. Prevalence of orthodontic post-treatment relapse in general is quite high, according to Sheibani et al., within 52 of 200 cases (26%), minimally 500 patients was treated, and the prevalence of relapse was 61.5% [32]. Even though, relapse occurrence is able to be prevented, so the aims of orthodontic treatment can be achieved. Tanya et al., have investigated the remodeling of the alveolar bone and related periodontal structures during orthodontic relapse in rat molars. The right first molars of 35 maxillary Wistar rats were moved mesially by a fixed orthodontic appliance for 10 days and the contra lateral molars served as controls. The appliances were removed and the six rats were sacrificed. The molars were allowed to relapse, and the remaining rats were sacrificed on day 1, day 3, day 5, day 7, day 14, and day 21 [33].

Orthodontic treatment is performed when pressure is applied on the teeth, then dental movement will occur within the alveolar bone around the teeth. Pressure on the crown of the tooth will be forwarded through the root of the tooth to the alveolar bone and periodontal ligament, then the alveolar bone surface, which gets pressure, will undergo resorption process and the opposite side will have a pull or apposition process. This is called the process of remodeling [1]. Alveolar bone remodeling is very important because it is the process of maintaining the balance of the dental support tissue [1], [2]. The remodeling process is used to maintain the thickness of bones and maintain the relationship between dental and alveolar bone in order to be relatively constant.

One way to prevent relapse is by retention. Retention is maintaining the new tooth movement in this position long enough to stabilize the correction [2]. Further, Nanda [2] also points out that a wide variety of causes of occurrence of relapses are the use of retainers, failure in removing the cause of the malocclusion, the inappropriate diagnosis and the failure in planning good care, interdigitation cups which are less normal, lateral and/or anterior jaw expansion, inappropriate size of the jaw and harmony, inappropriate axial inclination, failure in controlling the rotation, bad contact, and disharmony of the teeth size.

According to Indayani, factors which affect the occurrence of relapse are bone resorptions that experience the onset of movement of teeth nine times greater than the apotition bones, so it enables greater relapse. [3] To improve the apposition process by increasing osteoblast cell proliferation, fluoride is administered. Sakallioglu proves that giving high doses of fluoride can cause the increase of TGF-β 1 and TIMP/MMP and the average of TIMP/ TGF-β 1 [4]. Kebsch argues that the administration of fluoride increases the levels of osteocalsin in Wistar rats which have movement their teeth though statistically there is no significant difference [5]. According to Sakalliglu et al., fluoride increases bone turnover in which

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sodium fluoride enhances cell proliferation in the bone regulated by parathyroid hormone and bone growth factors [6].

Research on fluoride has been published, but the inhibition of relapse of orthodontic movement due in sodium fluoride (NaF) of Expression TGF-β1, Runx2, ALP and woven bone in molecular biology that occurs in the alveolar bone tissue after tooth movement still has not yet been explained thoroughly. Mechanisms of molecular biology in mechanical pressure received by teeth in orthodontic treatment are characterized by the presence of an inflammatory process in periodontal tissue. Inflammatory mediators will trigger biological processes in connection with resorption and apotition alveolar bone. The biological mechanisms that stimulate the alveolar bone resorption are physiologically related to cytokines. Cytokines are a group of mediators to tissue damage and are instrumental in the orthodontic tooth movement [7], [8]. Mechanical pressure also activates inflammatory cells, especially macrophages and neutrophils, to produce various chemical mediators, among others: Prostalglandins, Interleukin-1, Interleukin-6, tumor necrosis factor-alpha and the differentiation of osteoclasts by receptor activator of nuclear factor kappa B ligand and osteoprotegerin that can be identified on examination of gingival crevicular fluid [9], [10] Subsequently, the mechanical pressure stimulates the occurrence of alveolar bone resorption [19]. Bones resorption are marked with increased osteoclast activity controlled by cytokines i.e. IL-1, IL-6 and TNF-λ, which stimulate osteoclastogenesis in bone resorption through receptor activators NF-kβ ligand (RANKL), NF-kβ (RANK) and osteoprotegerin (OPG). Recent research indicates that OPG is RANKL inhibitor and inhibits the bond between RANKL and RANK, so it inhibits osteoclastogenesis [11], [12].

ALP released by osteoblasts can increase the apposition process [11]. Besides, it also happens to vascular changes that cause the migration of leukocytes out of capillary blood vessels as a result of vascular endothelial growth (VEGF) increase, followed by an acceleration average area of woven bone. Increased VEGF can be caused by Hypoxya-1λ-inducible factor (HIF-1λ) in synergy with runt-related transcription factor 2 (Runx-2) [13]. TGF-β1 is a cytokine together with BMP-2, which goes through the Smad pathway which will regulate Runx-2. This marker then stimulates mesenchymal stem cell (MSC) to change progenitor osteoblast. In administration, Runx-2 induces TGF-β 1 to further stimulate the differentiation and proliferation of osteoblast progenitors into active osteoblasts, which then leads to bone apposition [14].

Based on the background, it is important to identify the inhibition of relapse orthodontic tooth movement on sodium fluoride topical administration to the periodontal tissues through examination of the expression of TGF-β1, Runx-2, ALP, and comprehensive acceleration woven bone. Therefore, this study cannot be performed on humans; thus, this study is performed on male Wistar rats, using optimum strength of 10 g/cm2 by topical administration of gel sodium fluoride 11.75 ppm. Based on this conceptual background, the problem can be formulated as follows: Is there any increase in the number of osteoblast cells expressed by TGF-β1, Runx-2, ALP, and woven bone in male Wistar rats' teeth that experienced movement on the pulled side after sodium fluoride topical administrations. The purpose of the research is to explain the inhibition of relapses of orthodontic tooth movement in the provision of sodium fluoride through the expression of TGF β1, Runx2, ALP, and acceleration of the average area of woven bone. The significance of this study is to contribute to the development of scientific knowledge about the inhibition of relapse of orthodontic tooth movement by sodium fluoride topical administration.

II. Material and Methods

A. Rat Model of Orthodontic Tooth Movement

20 3-4 month-old male Wistar rats (weighted 0.2-0.3 kg) were used and divided into the following groups: control and experiment. The rats were healthy and divided into four groups, the names and characteristics are as follows:

Seven-day control group: Represented by rats C1, C2, C3, C1 and C5, which were given orthodontic treatment or did not receive fluoride topical application and were sacrificed after seven days of tooth movement.

Fourteen-day control group: represented by rats C6, C7, C8, C9 and C10, which obtained orthodontic treatment or did not receive fluoride topical application and were sacrificed after 14 days of tooth movement.

Seven-day experimental group: represented by rats E1, E2, E3, E4 and E5, which were administered orthodontic treatment and received fluoride topical application and were sacrificed after 7 days of tooth movement.

Fourteen-day experimental group: represented by rats E6, E7, E8, E9 and E10, which were administered orthodontic treatment and received fluoride topical application and were sacrificed after 14 days of tooth movement.

10 grams of orthodontic force were applied in the distal direction to upper incisor of male Wistar rats of a pre-fabricated closed stainless steel coil spring, measured with a Tension Gauge, (Ormco® Glendora, USA). The research protocol was approved by the Institutional Ethics Committee for Animal Experimentation of the Airlangga University.

B. Immunohistochemical Procedures

Mandibles were dissected, formalin-fixed, and decalcified with EDTA. Samples were dehydrated and paraffin-embedded. Tissue sections of less than 180 μm from the furcation of the distal root of upper incisor were stained for TGF β1, Runx-2, ALP and woven bone. Sections were dewaxed, blocked for endogenous hydrogen peroxidase, subjected to antigen retrieval, incubated with serum and monoclonal goat anti TGF β1, Runx-2, ALP antibody.

C. Statistical Analysis

The protein expressions (TGF β1,Runx-2, ALP and woven bone) in the tension side were different from one another and if duration of force (7 days and 14 days) affected the expression pattern, using Kolmogorov-Smirnov test, was

performed to show that data were normalized ($p>0.05$) and was followed by an independent t-test. It was used to compare to one another. The results were significant if $p<0.05$.

D.Histopathological Evaluation

The structures were histopathologically evaluated with an optical microscope (Nikon Eclipse E 600, Tokyo, Japan) and the regions of interest photographed with a digital camera (Nikon Coolpix 4500, Tokyo, Japan).

III. Results and Discussion

A. Description of Research Data

This study is experimental, using 30 male Wistar rats (Rattus norvegicus) aged 3-4 months, divided into three groups: (1) negative control group (K-) without movement and treatment of incisor teeth; (2) positive control group (K +) with movement of incisor teeth but no treatment; (3) treatment groups (P) with movement and treatment of incisor teeth by gel sodium fluoride topically. Observation of variables of each group was performed on day 7 and day 14.

TABLE I
Description of Variable Data on the Number of Osteoblasts in the Expressions of TGFb1, Runx2, ALP Expression, and Microscopic Appearance of Woven Bone

Group Experiment	K-Day 7 Mean ± SD	Day 14 Mean ± SD	K+Day 7 Mean ± SD	Day 14 Mean ± SD	P Day 7 Mean ± SD	Day 14 Mean ± SD
Expression of TGFβ1	2.8 ± 1.304	3.4 ± 0.894	6.8 ± 1.483	10.8 ± 1.634	13 ± 2.121	14.6 ± 1.673
Expression of Runx2	2.2 ± 0.836	2.6 ± 0.896	7 ± 1.732	11.6 ± 1.140	13.2 ± 2.588	15.6 ± 2.074
Expression ALP	6.2 ± 1.788	7.6 ± 1.342	11 ± 1.581	16.8 ± 2.280	13 ± 2.236	21.8 ± 4.382
Woven bone	1.9 ±0.625	1.9± 0.418	4 ± 0.707	5 ± 1.541	6.1 ± 0.418	8 ± 0.935

The results showed that immunohistochemical had a positive reaction toward TGF-β1, Runx-2, ALP, and woven bone with haematoxilin on the pull side.

Table I Mean and standard deviations (SD) of the results of the osteoblast cell count in the expressions of TGF β1, Runx2, ALP by immunohistochemical staining and woven bone with haematoxylin staining.

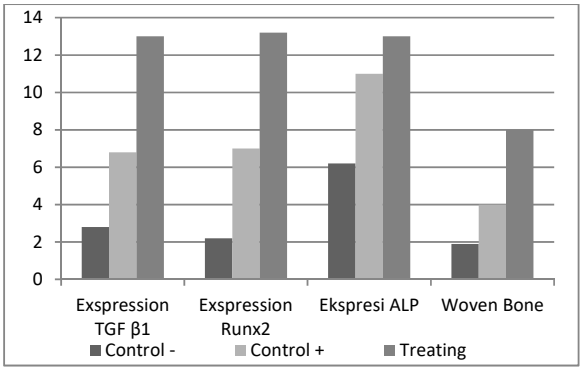

Fig. 1 Average number of osteoblasts on the expressions of TGFβ1, Runx2, ALP, and woven bone on day 7

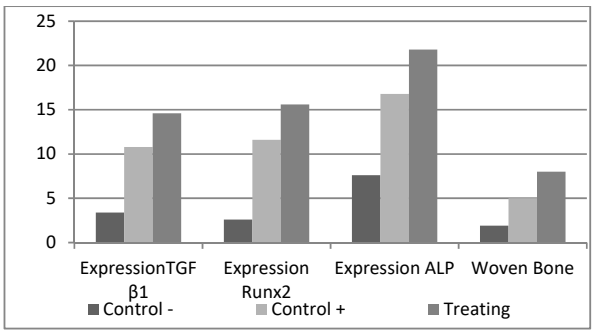

Fig. 2 Average number of osteoblasts on the expressions of TGFβ1, Runx2, ALP, and woven bone on day 14

Average data description and standard deviation (SD) of the expressions of TGFβ1, Runx2, ALP by histochemical and haematoxilin stainings, and woven bone on day 7 are presented in Fig. 1, which indicates the lowest average of woven bone at 11.75 ppm by giving sodium fluoride topically. Similarly, on day 14 in Fig. 2, shows the lowest average of woven bone at 11.75 ppm in administration of sodium fluoride.

Normal distribution of data is a requirement of some statistical analyses performed in this study. Before further analysis of data on the expressions of TGFβ1, Runx2 and ALP and woven bone, tested data homogeneity was tested first. Data homogeneity in this study is tested using the analysis of Box's Test. Table II presents that data on all groups already complete the assumption of homogeneity.

TABLE II
Homogeneity Test Data with the Box's Test

Box's M	72.090
Signification	0.244

Based on Table II, the value of 0.244 is significant, which is greater than 0.05, meaning that the expressions of TGFβ1, Runx2, ALP and woven bone groups have already completed the assumption of homogeneity. Then, normal distribution is tested for all variables. Fig. 3 presents the test results of the normal distribution of the variable count results of osteoblast cells to the expressions of TGF β1, Runx2, and ALP by immunohistochemical and woven bone by haematoxilin using QQ Plot for data analysis. The scatter plot in Fig. 3 appears likely to form a straight and diagonal line.

Beside visually, normal distribution test can also be done by calculating the value of Z. Based on the calculation of the test statistic Z values, 0.566667 is obtained. Thus, the value of the test statistic Z is greater than 0.05, meaning that the data TGFβ1, Runx2, and ALP expression by immunohistochemical staining and staining haematoxilin woven bone have met the

normal assumption, so it can be continued to the next analysis stage, namely one-way ANOVA analysis.

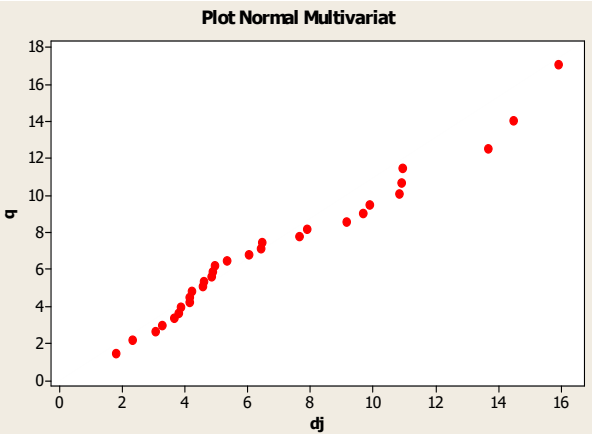

Fig. 3 Test results using normal distribution of data Plot QQ

B. Different Test Variables between Study Groups

Different test variables between the groups in this study are made on the calculation of the results of the examination of immunohistochemistry in osteoblast cells which give positive results toward TGFβ1, Runx2, and ALP expression by immunohistochemical staining and woven bone staining haematoxilin.

Based on the results of distribution normality test research data, different tests of variables between groups for variables TGFβ1, Runx2, ALP and woven bone are performed by one-way ANOVA test. Once the data are statistically tested by using test Wilks' lambda, the figure of 0.007 is gained, which is less than 0.05. It means that there are different effects between the groups.

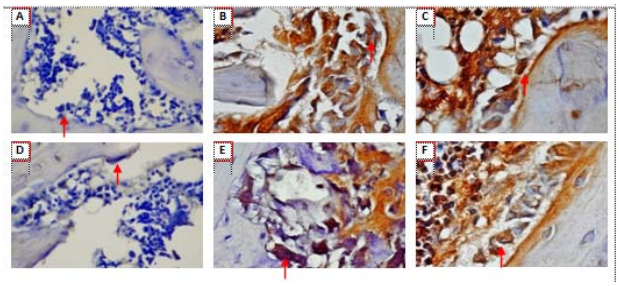

Fig. 4 Expression of TGF β 1 on osteoblasts alveolar bone cell is marked with red arrow. A is the negative control group (K-), B is the positive group (K +), C is the treatment group (P) by administration sodium fluoride 11.75 ppm on day 7, D is the negative control group (K-), E is the positive control group (K +), F is the treatment group (P) by administration sodium fluoride 11.75 ppm at day 14, with 1000X magnification

C. The Results of Immunohistochemical Examination of the Expression of TGF β 1

Positive expression data of TGF β 1 are obtained from the observation of osteoblast cells by immunohistochemical methods in the negative control group (K-), positive control (K +), and the treatment group (P) as a result of the administration of 11.75 ppm sodium fluoride topical application traction area. Here is a picture that presents the expression of TGF β 1 on osteoblasts alveolar bone on day 7 and on day 14.

Fig. 4 presents the overview of osteoblasts alveolar bone TGF β1 expression of positive reactions (brown) showing TGF β1 reaction with monoclonal antibodies (anti-TGF β1). The figure indicates that A and D (brown) are fewer than B, C, E and F.

TABLE III
DESCRIPTION OF THE MEAN, STANDARD DEVIATION (SD), AND BETWEEN THE DIFFERENT TEST GROUPS TGF B1 EXPRESSION IN THE NEGATIVE GROUP (K-), POSITIVE GROUP (K +) AND THE TREATMENT GROUP (P) BY ADMINISTRATION SODIUM FLUORIDE 11.75 PPM

Group		Average ± SD	F	P
Negative control	Day 7	2.8 ± 1.304		
	Day 14	3.4 ± 0.894		
Positive control	Day 7	6.8 ± 1.483	50.659	0.000*
	Day 14	10.8 ± 1.643		
Treatment	Day 7	13 ± 2.121		
	Day 14	14.6 ± 1.673		

Description * significant at α = 0.05

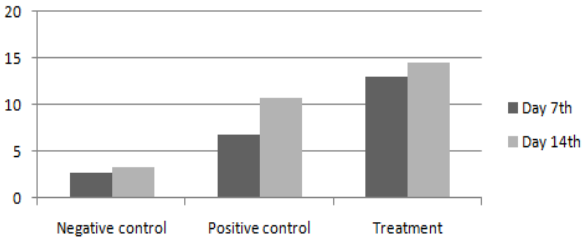

Fig. 5 Mean TGF β 1 expression between K (-), K (+), and the treatment on day 7 and day 14

The results of the one-way ANOVA analysis show a significant difference ($p<0.05$) between the negative control group (K-) and positive control group (K +) as well as the treatment group. The results show administration of sodium fluoride 11.75 ppm on the pull area can cause increase in mean expression of TGF β1 in osteoblast cells significantly compared with the positive control group but still higher when compared with the negative group (K-), as shown in Fig. 5.

D. The Results of Immunohistochemical Examination of the Expression of Runx2

Runx2 positive expression data derived from observations of osteoblast cells by immunohistochemical methods in the negative control group (K-), positive control (K +), and the treatment group (P) as a result of administration of sodium fluoride 11.75 ppm on the pull region. Fig. 6 shows the Runx2 expression in osteoblasts alveolar bone on day 7 and day 14.

Fig. 6 shows alveolar bone osteoblasts Runx2 expression (brown) gives a positive reaction indicating Runx2 reaction with monoclonal antibody (anti-Runx2). The figure indicates that cells in pictures A and D (brown) are fewer than those in pictures B, C, E and F.

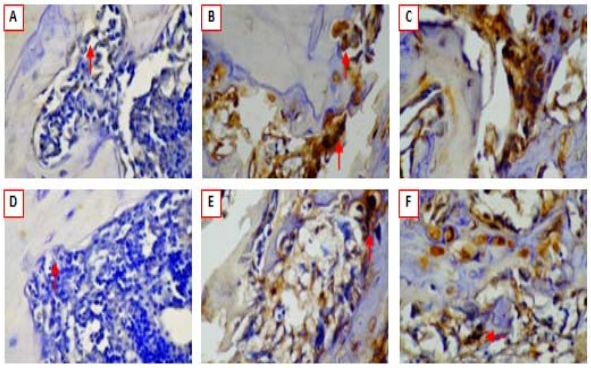

Fig. 6 Runx2 expression in alveolar bone osteoblasts cells is marked with red arrow. A is negative the control group (K-), B is the positive control group (K +), C is the treatment group (P) by administration of sodium fluoride 11.75 ppm on day 7, D is the negative control group (K-), E is the positive control group (K +), and F is the treatment group (P) by administration of sodium fluoride 11.75 ppm on day 14, with a 1000X magnification

TABLE IV
DESCRIPTION OF AVERAGE, STANDARD DEVIATION (SD), AND THE DIFFERENT TEST GROUPS RUNX2 EXPRESSION IN THE NEGATIVE GROUP (K-), POSITIVE GROUP (K +) AND THE TREATMENT GROUP (P) BY ADMINISTRATION OF SODIUM FLUORIDE 11.75 PPM

Group		Average ± SD	F	P
Negative control	Day 7	2.2 ± 0.836		
	Day 14	2.6 ± 0.894		
Positive control	Day 7	7 ± 1.732	56.650	0.000*
	Day 14	11.6 ± 1.140		
Treatment	Day 7	13.2 ± 2.588		
	Day 14	15.6 ± 2.074		

Description * significant at α = 0.05

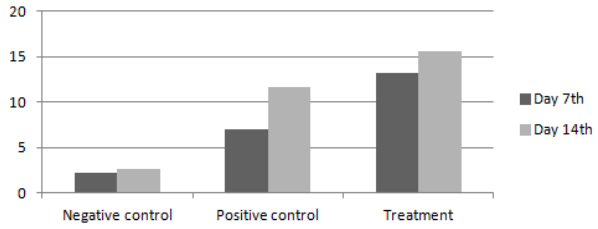

Fig. 7 Mean Runx2 expression between K (-), K (+), and the treatment on day 7 and day 14

The results of the one-way ANOVA analysis show a significant difference ($p<0.05$) between the negative control group (K-), positive control group (K +) as well as the treatment group. The results show that administration of sodium fluoride 11.75 ppm on the pull region can increase the mean expression of Runx2 in osteoblast cells significantly compared with the positive control group but still higher when compared with the negative group (K-), as shown in Fig. 7.

E. The Results of Immunohistochemical Examination of the Expression of ALP

A positive expression data of ALP derived from observations of osteoblast cells by immunohistochemical methods in the negative control group (K-), positive control (K +), and the treatment group (P) as a result of administration of sodium fluoride 11.75 ppm in the pull region. Fig. 8 shows the ALP expression in alveolar bone osteoblasts on day 7 and on day 14.

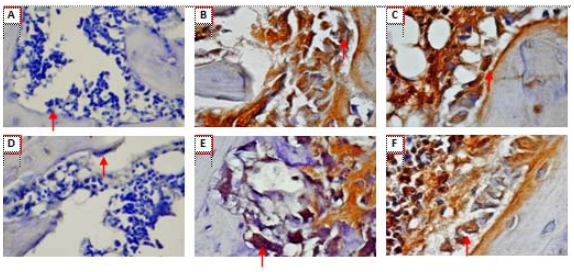

Fig. 8 The expression of ALP in alveolar bone osteoblasts cells are marked with a red arrow. A is the negative control group (K-), B is the positive control group (K +), C is the treatment group (P), D is the negative control group (K-), E is the positive control group (K +), and F is the control group treatment (P), with a 1000X magnification

Fig. 8 indicates a microscopic view of alveolar bone osteoblasts ALP expression (brown) gives positive reactions shown by ALP reaction with monoclonal antibody (anti-ALP). The figure indicates A and D brown fewer than the B, C, E and F.

TABLE V
MEAN, STANDARD DEVIATION (SD), AND BETWEEN THE DIFFERENT TEST GROUP ALP EXPRESSION IN THE NEGATIVE GROUP (K-), POSITIVE GROUP (K +) AND TREATMENT GROUP (P)

Group		Average ± SD	F	P
Negative control	Day 7	6.2 ± 1.788		
	Day 14	7.6 ± 1.342	27.781	0.000*
Positive control	Day 7	11 ± 1.581		
	Day 14	16.8 ± 2.280		
Treatment	Day 7	13 ± 2.236		
	Day 14	21.8 ± 4.382		

Description * significant at α = 0.05

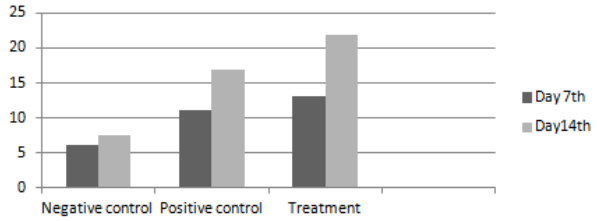

Fig. 9 Average ALP expression between K (-), K (+), and the treatment on day 7 and day 14

The results one-way ANOVA analysis showed significant differences ($p <0.05$) between the negative control group (K-), which was not given the strength of the orthodontic, and the positive control group (K +) by orthodontic force and not given sodium fluoride topical, as well as the treatment group which were given the power of orthodontic by administration of sodium fluoride topically. The results show administration of sodium fluoride 11.75 ppm topically can improve the traction area mean ALP expression in osteoblast cells that

significantly compared with the positive control group, but were still higher when compared with the negative group (K-), as in Fig. 9.

F. Results of the Examination to the Acceleration of Comprehensive Haematoxilin Woven Bone

Woven bone mage data derived from observations of osteoblast cells by staining haematoxilin the negative control group (K-), positive control (K +), and the treatment group (P) as a result of Administrations of sodium fluoride 11.75 ppm topically to pull the region. Fig. 10 shows depict woven bone on osteoblast cells on day 7 and day 14. Fig. 10 shows that A and D are less than B, C, E and F.

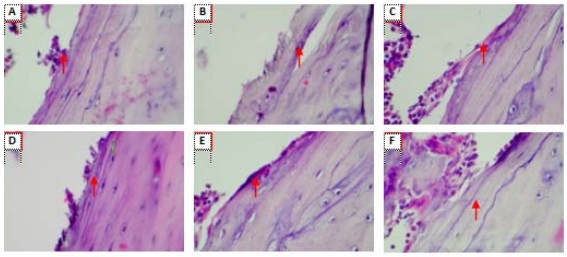

Fig. 10 Images depict woven bone on the alveolar bone marked with red arrow. A is the negative control group (K-), B is the positive control group, C is the treatment group (P) by administration of sodium fluoride topical 11.75 ppm on day 7, D is the negative control group (K-), E is the positive control group, and F is the treatment group by administration of sodium fluoride 11.75 ppm topically on day 14 with a 1000X magnification

TABLE VI
MEAN, STANDARD DEVIATION (SD), AND BETWEEN THE DIFFERENT TEST GROUPS WOVEN BONE PICTURE IN NEGATIVE GROUP (K-), POSITIVE GROUP (K +) AND THE TREATMENT GROUP (P) IN ADMINISTRATION OF SODIUM FLUORIDE 11.75 PPM (M)

Group		Average ± SD	F	P
Negative control	Day 7	1.9 ± 0.652		
	Day 14	1.9 ± 0.418		
Positive control	Day 7	4 ± 0.707	38.225	0.000*
	Day 14	5 ± 1.541		
Treatment	Day 7	6,1 ± 0,418		
	Day 14	8 ± 0.935		

Description * significant at α = 0.05

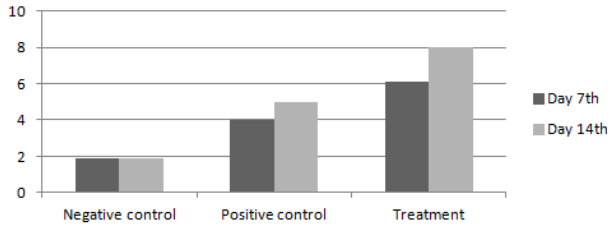

Fig. 11 Comparison of average woven bone for K-, K +, and Treatment on day 7 and day 14

The results of the one-way ANOVA analysis show a significant difference ($p<0.05$) between the negative control group (K-), positive control group (K +) and the treatment group. The results show that administration of sodium fluoride 11.75 ppm can improve the traction area average woven bone expression in osteoblast cells significantly compared with the positive control group but still higher when compared with the negative group (K-), as shown in Fig. 11.

The different test variables between the study groups on day 7 and day 14 are presented in Table VII.

TABLE VII
RESULTS OF DIFFERENT TEST VARIABLES BETWEEN THE RESEARCH GROUPS FOR DAY 7 AND DAY 14 USING T-TEST

Variable	P
TGF β 1	0.2380
Runx2	0.2518
ALP	0.0095*
Woven Bone	0.2704

Results of the comparison of test variables between the groups on day 7 and day 14, show there are no significant differences in the ALP variable, or the variables TGF β 1, Runx2, ALP, and woven bone.

IV. DISCUSSION

Experimental research on the relapse inhibition of orthodontic tooth movement used sodium fluoride 11.75 ppm in the form of topical gel application as a treatment given the groove upper incisor teeth Wistar rats with movement by the strength of 10 g/cm^2, regional tug on osteogenesis mechanism to prevent relapse. A dose of 11.75 ppm sodium fluoride was obtained from the calculation of equalization between the optimal dosages of sodium fluoride topical application used by humans, which is 400 ppm [15], [16] at a dose of experimental animals, in this case, Wistar rats.

The active substance sodium fluoride 11.75 ppm in the form of gel topically was given to the gingival sulcus left upper incisor teeth Wistar rats included in Plasma membrane cell through an active transport by means of ion channel passes [17]. Given the nature of sodium fluoride is a dissolution of Na + ions and ion F^-, F^-interacting with $NH3^+$amino acid group that would interact with the protein in macrophages, thereby increasing the expression of the markers in this study. After passing through the ion channel, Fluor entered the cytoplasm passing lane marker MAPK and might serve to alter gene expression.

In this study, three groups of rats are: (1) negative control group, which is the group with no movement gear and no sodium fluoride; (2) positive control group; a group of teeth with movement but without sodium fluoride, and (3) treatment groups: group gear with movement and sodium fluoride. This study used the power of orthodontic pressure and treated in the form of sodium fluoride gel with a dose of 11.75 ppm, for 7 days and 14 days, to explain the biological cellular and molecular response to the treatment of sodium fluoride in the process of osteogenesis as protective gear relapse.

Orthodontic strength is used to move the teeth to produce a variety of reactions from the tooth in itself or from the contiguous tissue, including changes in the expression of hundreds of genes in cells contained in periodontal tissues. In

tooth orthodontic movement, there are balancing between alveolar bone resorptions and deposition of new bone that involves a physical response, cellular, biochemical and molecular around periodontal tissue surrounding the root such as neurotransmitter gene expression, signaling molecules, extracellular matrix components, cytokines, chemokines, growth factors, transcription factors, protease, a protein contained in the mineralized bone remodeling. Cellular and molecular responses as a result of orthodontic tooth movement in the area pull on alveolar bone are evaluated through observation of osteoblasts. This research method used immunohistochemical techniques in the calculation of cell number expression of variables: TGF β1, Runx2, ALP, and woven bone to administration of sodium fluoride in the form of topical gel of 11.75 ppm.

Based on statistical analysis, data from this study are homogeneous with normal distribution in each group that showed the significant difference $p = 0.244$ ($p > 0.05$) as in Table VII, making it eligible for the next statistical test.

The results showed that the average description expression of TGFβ 1 in the treatment group is greater than the positive control group and negative control group, while the expression of TGFβ 1 in the positive control group is greater than the negative control group. This is because TGFβ 1 stimulates and activates macrofag and limfosit to clean debris periodontal tissue due to the orthodontic strength [18]. TGFβ 1 is also a pro-inflammatory cytokine and anti-inflammatory cytokines [9]. Thus, the negative control group has the lowest number since the incisive teeth in the group is with no movement, so there is no inflammatory process which leads to the absence of increase in the expression of TGFβ 1. One-way ANOVA test results on the examination of the expression of TGF β 1, as shown in Table III, show significant difference ($p < 0.05$), meaning that there is a significant difference between the negative control group, positive control group and treatment group. The results show the administration of sodium fluoride can increase the expression of TGFβ 1 significantly. This is similar to the results of research by Huang et al. that found Marmot gear-movement by administration fluoride with a dose of 40 mg/L can increase TGF β 1 [14]. Administration of NaF in some studies can increase cell proliferation, including osteoblast; thus, TGF β 1 in this study, one of which is produced by osteoblasts, increases the osteoblasts in line with the increased expression of TGF β 1. TGF β 1 is included in the growth factor which is described as a molecule that has the ability to stimulate cell growth, proliferation and differentiation of cells, so it can regulate cellular processes involving the development and maintenance of homeostasis tissue and the wound healing process. In administration, TGF β1can regulate the expression of collagen type 1, hold precursor osteoblasts, stimulate the differentiation of cells, and increase matrix protein in the production of bone. TGF 1 β is proved to increase during the observation of orthodontic movement in the pull area [19]. The observation of this study at day 7 and day 14 in the administration of sodium fluoride topical shows improvement but no significant difference. This is because sodium fluoride can increase osteoblast, so that TGF β 1 producing osteoblasts also increases [20], [21].

The results showed that the average description of Runx2 expression is greater in the treatment group than the positive control group and negative control group, while the expression of Runx2 in the positive control group is greater than the negative control group. This is in line with research by Stein et al., which showed Runx protein function in the process of osteogenesis in mammals. Runx2 is an primary osteogenic, while Runx1 and Runx3 is expressed in bone cells and assists in the development and differentiation of bone cells [22]. They conclude that Runx2 controls integration, organization, and regulatory factors for bone gene expression. Likewise, research by Fujita et al. confirms the role of Runx2 in osteoblast differentiation and mesenchymal stem cell [23]. The research studies show that Runx2 is a transcription factor that is important in the process of osteogenesis on orthodontic tooth movement. One-way ANOVA test results shown in Table IV show a significant difference ($p<0.05$), meaning that there is a significant difference between the negative control group, positive control group as well as the treatment group. The results show that the administration of sodium fluoride can increase the expression of Runx2 significantly. This is because it can increase the metabolism of bone turnover and bone cell proliferation [24]. Some factors affecting bone turnover are hormonal factors, cytokines and mechanical stimulation. These factors affect the quantity and quality of the resulting network [25]. The observation of this study on day 7 and day 14 on the administration of sodium fluoride topical shows improvement but no significant difference. This is because sodium fluoride can increase osteoblast; besides, Runx2 is a factor that is expressed transcription earliest, so on day 14, there is no sharp increase [26].

The results of the average description show that ALP expression is greater in the treatment group compared with the control group, positive and negative control groups, while the ALP expression in the positive control group is greater than the negative control group. This is because the ALP marker is associated with the activity of osteoblasts [27]. In other side, hydrolyzes nonorganic pyrophosphate as inhibitor of mineralization process [28].

One-way ANOVA test results shown in Table V show a significant difference ($p < 0.05$), meaning that there is a significant difference between the negative control group, positive control group as well as the treatment group. This is because fluoride can increase total serum ALP [29]. The observation of this study on day 7 and day 14 on the administration of sodium fluoride topical shows improvement and there is a significant difference since ALP activity reaches the peak on day 14 [30].

The results showed that the average comprehensive description woven bone is greater in the treatment group compared with the control group positive and negative control group, while the area of woven bone in the positive control group is greater than the negative control group. This may imply that the result of administration of sodium fluoride 11.75 ppm causes acceleration because extensive woven bone sodium fluoride increases bone-forming cell proliferation

increases bone formation which further improves bone apposition. The formation of intramembrane bone produces woven together with lamellar bones forming the primary bone [19]. The results of this research observation on day 7 and day 14 on the administrations of sodium fluoride topical show improvement but no significant difference. This is because the woven bone on days 7 and 14 is still in the process of formation. Bone woven formation reaches the peak on around day 30. After day 30, woven bone formation decreases due to an increase in the formation of osteocytes. In the event of an increase in the number of comprehensive osteocytes, while woven bone markers decrease. Contradictorily, Runx2 decreases while increasing Osterix. Giving Fluor also increases the expression of osteogenic markers resulting in the formation of bone in the form of woven bone also increases [31]. Therefore, there is no significant difference on day 7 and day 14; it can be said that the acceleration of the average area of woven bone occurs on day 7.

V. Conclusion

1. Administration of sodium fluoride application on orthodontic tooth movement effectively enhances the expression of TGF β 1, Runx2, ALP, and the average area of the region woven bone pull significantly from the group that is not given sodium fluoride.
2. Administration of sodium fluoride on orthodontic tooth movement increases the expression of TGF β 1, Runx2, and the average area woven bone in the area but no significant pull on day 7 and day 14, except in the expression of ALP.
3. Acceleration average woven bone area increases on sodium fluoride topical administration was proved on day 7.

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