

FGF-2, MMP-8 and Integrin $\alpha 2\beta 1$ Expression in Periodontal ligament Remodelling Tension Area with Nanopowder *Stichopus hermanii* Application to Prevent Orthodontic Relapsing

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Abstract: Background: Orthodontic relapse tendency occurs 33–90 per cent after at least 10 years post-treatment. Orthodontic Relapsing is the return, following orthodontic correction, at the end of treatment. Relapse, a return to the original teeth position, caused by periodontal, occlusal, soft tissue factor and growth. Nanopowder *Stichopus hermanii* have various active ingredient such as protein, collagen, flavonoid, chondroitin sulphate, cell growth factor, EPA DHA, that might have role to FGF-2, MMP-8, Integrin $\alpha 2\beta 1$ in periodontal ligament remodelling tension area in reducing orthodontic relapsing. Objectives: The aim of this study is to investigate application of Nanopowder *Stichopus hermanii* to prevent relaps orthodontic by periodontal ligament remodelling tension area through FGF-2, MMP-8, and Integrin $\alpha 2\beta 1$ parameter. Material and Method: The experiment was held by Post Test Only Group design. Twenty four male Cavia Cobaya were divided into three groups. K(-) group as negative control group (without treatment), K(+) group as positive control group which were applied with relaps orthodontic forces, and the other groups P, were applied with relaps orthodontic forces and nanopowder *Stichopus hermanii* 3%. After treatment the cavia cobaya were sacrificed. FGF-2, MMP-8, and Integrin $\alpha 2\beta 1$ expression is examined with immunohistochemistry. Results: Application nanopowder *Stichopus hermanii* can reduce relaps until 30%. This study showed FGF-2 expression are 13.9 ± 2.6 ; 6.5 ± 1.4 ; 21 ± 4.6 ; MMP-8 expression means and SD in K(-), K(+), P are 3.75 ± 1.49 ; 14.88 ± 2.64 ; 9.13 ± 1 and Integrin $\alpha 2\beta 1$ expression are 5.25 ± 1.28 ; 12.88 ± 1.36 ; 16.50 ± 2.33 . There was significantly differences in group P compare to K(-) and K(+). Conclusion: Application of Nanopowder *Stichopus hermanii* have role in periodontal ligament remodeling tension area through increasing FGF-2, decreasing MMP-8, increasing Integrin $\alpha 2\beta 1$ and Collagen type 1 parameter to prevent relaps orthodontic until 30%.

Keywords: Nanopowder *Stichopus hermanii*, FGF-2, MMP-8, Integrin $\alpha 2\beta 1$, Relaps Orthodontic

1. Introduction

Relapse occurred at the end of active orthodontic appliance. There was a rapid relapse initially after appliance removal but after 3 days, and began to gradually decrease. After appliance removal, the teeth began to relapse in the direction of their original position [1]. The teeth began moved to tension area and produce periodontal remodeling. Instability or a tendency toward relapse should be anticipate. Patients should be

advised of potential for relapse prior treatment and the need to stay in long-term retention [2]. Beside retention, fibrotomy is also known for diminished relapse [3].

Many technique used to prevent orthodontic relapsing but there is no natural has been used for relapse orthodontics. *Stichopus hermanii* belong to the phylum Echinodermata, under the class Holothuridea. It was further divided into three subclasses namely Dendrochirota, Aspidochirota, and Apodacea. There are six orders under these subclasses,

named as *Aspidochirotida*, *Apodida*, *Dactylochirotida*, *Dendrochirotida*, *Elasipodida* and *Molpadiida* [4]. *Stichopus hermanii* have been well recognized as traditional medicine in Chinese. The Chinese have long regarded sea cucumbers as a general health tonic, beneficial for treating tendonitis and arthritis, and as an aphrodisiac, among many other medicinal claims. The Chinese also consider *Stichopus hermanii* as culinary [5]. Malaysian value *Stichopus hermanii* for hypertension, asthma, rheumatism, burns, impotence and constipation treatment [6] and also for wound healing, treatment of stomach ulcers and as a painkiller, and as culinary delicacies [5]. The Japanese eat *Stichopus hermanii* row, nibble on their pickled intestines over drinks and eat their dried gonads as a special treat [7].

Previous research showed that *Stichopus hermanii* can increase Collagen type 1 expression in relaps orthodontic. *Stichopus hermanii* can act as anticandidal [8],[9] *Stichopus hermanii* treated wounds and stimulation tissue regeneration. [10]. The other study show that studies have shown that the extract of *Stichopus* species also affects viability or proliferation of human fibroblasts and osteoclast cells in a negative manner [10].

2. Material and Methods

The study is true experimental laboratories with completely randomized control group post test only design. Ethical permission was obtained from Ethics and Scientific Research Committee of Experimental Animal Use in Dentistry Faculty Airlangga University. Twenty four male guinea pigs (*Cavia cobaya*) aged 2,5 months and weighed 200-300 grams. The guinea pigs, fed with a standard pellet diet and tap water ad libitum, were randomly divided into three equal groups. The materials used were 3% and 3,5% *Stichopus hermanii*, 10% ketamine injection as anesthetic drug, a dose of 0.1-0.2 ml/kg for acepromazine 0.5 ml, 10% buffered formalin, and cotton.

2.1. Nanopowder *Stichopus hermanii* Preparation and Quantitative Analysis Content

Stichopus hermanii were used in this study from coastal regions around Sumenep, East Java Indonesia. *Stichopus hermanii* was cleaned by making a longitudinal incision 3-5 cm on the ventral side of *Stichopus hermanii* without damaging the internal organs using scalpel. *Stichopus hermanii* was dried by ovens, blender, made nanopowder by using High Energy Milling method. SEM and TEM pictures reveal the morphology and particle size of nanopowder *Stichopus hermanii*. Quantitatives analysis of *Stichopus hermanii* active ingredients use spectrophotometry to examine flavonoid, gas chromatography to examine EPA and DHA, and HPLC to examine chondroitin sulphate.

2.2. Preparation of Orthodontic Relapsing

Orthodontic relapsing forces was produced by using separator rubber application with separating plier in mesial left insisivus maxilla *cavia cobaya* 14 days and after day 15

separator was removed for 7 days for producing orthodontic relapse orthodontic. Separator forces was 0,0474 kN, measured by autograph. Biometric orthodontic relapsing was measured by caliper.



Figure 1. Biometric orthodontic relapsing measure.

2.3. Preparation and Applied *Stichopus hermanii* Gel

Stichopus hermanii gel 3% was made from 0,3 gr *Stichopus hermanii* powder was diluted with NaCMC 2% in DMSO 5% until 10 ml. *Stichopus hermanii* gel was applied in gingival sulcus with insulin syringe 0,025 ml twice per day.

The procedure of this study was began with acclimatization of animals for 48 hours. Next, Guinea pigs were divided into 4 groups. K(-) group as negative control group (without treatment), K(+) group as positive control group which were triggered orthodontic tooth movement by using elastic separator, the force was 0,0474 kN, measured with gauge during experiment and the other groups P1, were applied with both orthodontic forces and *Stichopus hermanii* 3% in 14 days and released the forces for 7 days. *Stichopus hermanii* gel was applied in gingival sulcus with insulin syringe 0,025 ml once per day.

The research was conducted in Biochemistry Laboratory Medical Faculty of Airlangga University. The guinea pigs were monitored during the experiment, and all of the groups were sacrificed on the twenty first day of the experiment. The maxillary insisivi teeth were dissected and placed in 10% buffered formalin. Afterwards, histological section were prepared with FGF-2, MMP-8, integrin $\alpha 2\beta 1$ immunohistochemistry as periodontal remodeling marker, and then observed by using a microscope.

The photos were taken to measure the FGF-2, MMP-8, integrin $\alpha 2\beta 1$ expression in periodontal ligament tension area was observed seen on the microscope with an enlargement 400x. Each histological section was observed and calculated as many as three times in the field of view.

Finally, the data were statistically measured by using Statistical Package for the Social Science (SPSS) version 20 program. The research data result tabulated and planned to analyze by descriptive statistic test, normality distribution test to know if the data that obtained come from population with normal distribution, ANOVA test (analysis of varians) to analyze the difference of each variable compared with control. Then the data were tested with LSD Test ($p < 0.05$).

3. Result

The aim of this study is to investigate application of Nanopowder *Stichopus hermanii* to prevent relaps orthodontic by periodontal ligament remodelling tension area through FGF-2, MMP-8, and Integrin $\alpha 2\beta 1$ parameter.

3.1. Biometric Orthodontic Relapsing

Table 1 and figure 2 below show the biometric orthodontic relapsing.

Table 1. Descriptive Mean and Standard Deviation of Biometric orthodontic relapsing (mm).

Group	Mean \pm Standard Deviation
K(-)	0.00 \pm 0.00
K(+)	0.09 \pm 0.02
P	0.06 \pm 0.02

Table 1 shows means and SD in K(-), K(+), P are 0,00 \pm 0,00 ; 0,09 \pm 0,02; 0,006 \pm 0,02. Then the data were tested with

normality test, homogeneity test and show the data was homogen and have a normal distribution. ANOVA test ($p=0.05$) for the biometric orthodontic relapsing Cavia Cobaya applied with *Stichopus hermanii* showed significantly differences.

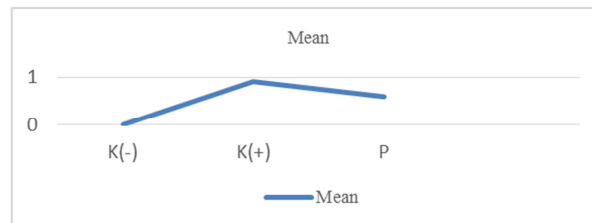


Figure 2. Line chart mean biometric orthodontic relapsing.

3.2. FGF-2 Expression

FGF-2 expression mean and standard deviation as seen as table 2 and figure 3 below.

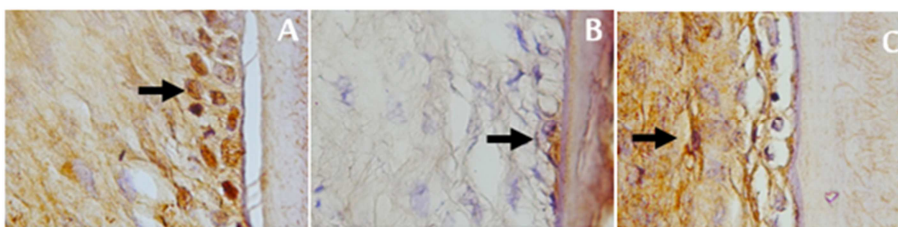


Figure 3. FGF-2 expression in K(-) group (A), K(+) group (B), P group (C).

Table 2. Descriptive Mean and Standard Deviation of FGF-2 expression (cell/view of field).

Group	Mean \pm Standard Deviation
K(-)	13,9 \pm 2,65
K(+)	6,5 \pm 1,41
P	21 \pm 4,61

Table 2 show FGF-2 means and SD in K(-), K(+), and P are 13,9 \pm 2,65 ; 6,5 \pm 1,41; 21 \pm 4,61, P group have the highest FGF-2 expression. Then the data were tested with normality test, homogeneity test and show the data was homogen and have a normal distribution. ANOVA test ($p=0.05$) for the

expression of FGF-2 in orthodontics relapsing. Cavia Cobaya applied with Nanopowder *Stichopus hermanii* showed significantly differences. With the Tukey HSD test, showed that MMP-8 expression was significantly increased in P compare to K(+) and K(-).

3.3. MMP-8 Expression

MMP-8 expression mean and standard deviation as seen as table 3 and figure 4 below.

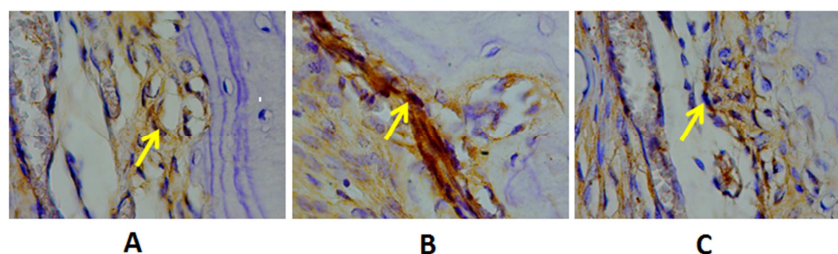


Figure 4. MMP3 expression in K(-) group (A), K(+) group (B), P group (C).

Table 3. Descriptive Mean and Standard Deviation of MMP-8 expression (cell/view of field).

Group	Mean \pm Standard Deviation
K(-)	3.75 \pm 1.49
K(+)	14.88 \pm 2.64
P	9.13 \pm 1.55

Table 3 show MMP-8 means and SD in K(-), K(+), P are 3,75 \pm 1,49 ; 14,88 \pm 2,64; 9,13 \pm 1,55. Then the data were tested with normality test, homogeneity test and show the data was homogen and have a normal distribution. ANOVA test ($p=0.05$) for the expression of MMP-8 in relaps orthodontics Cavia Cobaya applied with Nanopowder *Stichopus hermanii*

showed significantly differences. With the Tukey HSD test, showed that MMP-8 expression was significantly increased in P compare to K(+) and K(-).

3.4. Integrin $\alpha 2\beta 1$ Expression

Integrin $\alpha 2\beta 1$ expression mean and standard deviation as seen as table 4 and figure 5 below

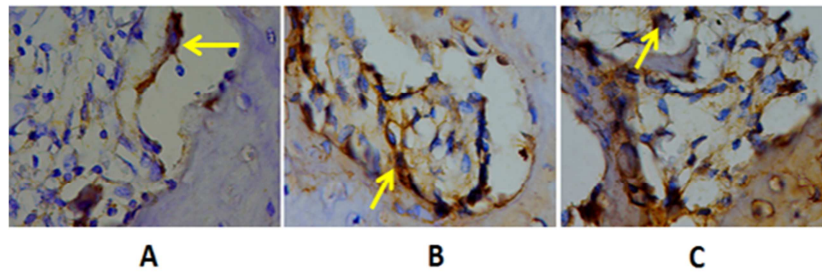


Figure 5. Integrin $\alpha 2\beta 1$ expression in K (-) group (A), K(+) group (B), P group (C).

Table 4. Descriptive Mean and Standard Deviation of Integrin $\alpha 2\beta 1$ expression (cell/view of field).

Group	Mean \pm Standard Deviation
K(-)	5,25 \pm 1,28
K(+)	12,88 \pm 1,36
P	16,55 \pm 2,33

Table 4 show the highest Integrin $\alpha 2\beta 1$ expression means and SD in K(-), K(+), P is in P1 16,55 \pm 2,33. After the data were tested with normality test, homogeneity test and show the data was homogen and have a normal distribution, then ANOVA test ($p=0.05$) was used for the expression of Integrin $\alpha 2\beta 1$ in relaps orthodontics Cavia Cobaya applied with Nanopowder *Stichopus hermanii*. The result showed significantly differences in all groups. With the Tukey HSD test, showed that Integrin $\alpha 2\beta 1$ expression was significantly increased in P compare to K(+) and K(-).

4. Discussion

One major reasons is the gingival and periodontal tissues are affected by orthodontic tooth movement and require time for reorganization when appliance are removed [12], [2]. Stretching of supraalveolar gingival fibres, the transseptal fibres, in particular, has been suggested as the cause of relapse [13].

The aim to this study was to investigate the periodontal ligament remodeling by *Stichopus hermanii* showed FGF-2 means and SD in K(-), K(+), P are 13,9 \pm 2,65 ; 6,5 \pm 1,41; 21 \pm 4,61. It means in relaps orthodontic, there are increasing fibroblast activity in P compare to K (-) and K (+). By giving Nanopowder *Stichopus hermanii* 3% concentration in gingival sulcus tension site, FGF-2 activity was significant increase.

When orthodontic tooth movement occurs, cells in the periodontal ligament response differently to tensile and to compressive strains, which predominantly catabolic tissue changes at sites under compression and predominantly anabolic activity at sites under tension area. Tensional forces of sufficient magnitude will stretch the plasma membrane increasing the channel diameter and the flow of ions implied the electrical conductivity of the membrane is increased. This activates intracellular signalling eliciting cellular responses [14], [15]. This causes cell deformation, which produce

activation of fibroblasts and osteoblasts in the periodontal ligament and osteocytes within bone. Combination of remodelling of the periodontal and remodelling of the alveolar bone makes the tooth to move [16]. Differently when relaps occurred and tension area losing strain and implied decreasing electrical conductivity. Remodelling of the periodontal ligament is essential for orthodontic tooth movement [17], [14]. The periodontal contribution to the equilibrium that normally controls tooth position [12].

Relapse in cavia cobaya models occurs rapidly. The left first insisivus compressed towards the distal side during 14 days orthodontic tooth movement and relapsed toward the mesial side. There was a rapid relapse initially following 7 days appliance removal. Increasing FGF-2 expression by applying *Stichopus hermanii* during relapse period means there is increasing fibroblast that manner as a collagen production that is important for periodontal ligament remodeling begins. Periodontal ligament plays a central role for remodeling periodontal ligament dan alveolar bone.

Periodontal Ligament is a fibrous structure with tensile strength and elasticity, connecting the tooth to the alveolar bone. When mechanical forces load, the Periodontal ligament fibers will be repelled from one another and then rebound as simulating an elastic effect. When the initial phase of orthodontic tooth movement in which the tooth is displaced within the periodontal ligament space, the inflammation occurred and affect the viscoelastic properties of both the bone and the periodontal ligament. There is decreasing tensile strength of the collagen bundles as a result of release of matrix metalloproteinases (MMPs) and other catabolic agents which disrupt the cross-linkages and molecular integrity of the extracellular matrix, and the hydrodynamic damping effect of the periodontal ligament is decreased, while the elasticity of the bone is increased [15]. The same way occurred when the tooth move back to the original position or orthodontic relapsing.

Stichopus hermanii contain various active ingredient such as hyaluronic acid, chondroitin sulphate, cell growth factor, EPA DHA, flavonoid [4]. In this research showed that there is decreasing MMP-8 when we apply Nanopowder *Stichopus hermanii* it means increasing the tensile strength of the collagen increase and implied bone remodeling. Nanopowder

Stichopus hermanii 3% contain flavonoid are known as strong inhibitors of metaloproteinases degrading matrix proteins. The increased level of collagen could result from inhibition of metaloproteinases activity[18].

The applied orthodontic forces compress when orthodontic tooth movement, stretching occurred in tension area, alter the collagen fibers and fluid within the periodontal ligament space, thus disrupting the configuration of the extracellular matrix proteins, induce FGF-2 that can activate the fibroblasts via integrins and focal adhesion[12]. Some of these activate molecules and induced signals generated the expression of genes encoding several proteins and enzymes essential for the remodelling of the extracellular matrix of the periodontal ligament. These proteins include collagen and fibronectin, and the enzymes include MMPs, serine proteases, aspartate proteases, and cysteine proteases that degrade and remodel the collagen and other macromolecules [15].

Flavonoids stimulated the activity of prolidase which catalyzes the final step of collagen and plays an important role in collagen biosynthesis through integrin-mediated signaling [18]. In this research, showed that integrin $\alpha 2\beta 1$ have increased with Nanopowder *Stichopus hermanii* application Integrin $\alpha 2\beta 1$ means and SD in K(-), K(+), P are $3,75 \pm 1,49$; $14,88 \pm 2,64$; $9,13 \pm 1,55$. Integrin $\alpha 2\beta 1$ expression was significantly increased in P compare to K(+) and K(-). Collagen production have important role for periodontal ligament remodeling in relaps orthodontic.

Stichopus hermanii also have heparan sulphate that modulate FGF-2 production and mediates a variety of physiological responses in development, cell growth, cell migration, and wound healing. The effects of basic fibroblast growth factor-2 (FGF-2), which is known to modulate extracellular matrix (ECM) production of various cell types [19]. FGF-2 also plays role in inducing osteopontine expression, bone protein that important for bone remodeling so can prevent orthodontic relapsing [20]. Osteopontin directly modulates bone formation in the response to mechanical stress [21]. Bone formation could be resistant to resorption and countered the Orthodontic relapsing [22]. It is showed in biometric orthodontic relapsing that relapse reduce 30% with Nanopowder *Stichopus hermanii* application.

5. Conclusion

1. Application of Nanopowder *Stichopus hermanii* have role in periodontal ligament remodeling tension area through increasing FGF-2, decreasing MMP-8, and increasing Integrin $\alpha 2\beta 1$ to prevent relaps orthodontic until 30%.
2. Nanopowder *Stichopus hermanii* 3% contain flavonoid are known as strong inhibitors of metaloproteinases degrading matrix proteins so the collagen is increased and have heparan sulphate that modulate FGF-2 production with effects of basic fibroblast growth factor-2 (FGF-2), which is known to modulate extracellular matrix (ECM) production such as collagen. FGF-2 also plays role in inducing osteopontine

expression, bone protein that important for bone remodeling that can prevent orthodontic relapsing

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