**Stichopus hermanii** stimulation to Runx2 expression as Periodontal Remodeling Biomarkers to accelerate Orthodontic Tooth Movement

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**Abstract:** The aim of this study is to investigate **S.hermanii** stimulation to Runx2 expression in periodontal remodeling during orthodontic tooth movement. In the experimental method twenty four males Cavia Cobaya were divided into three groups. K(-) group as negative control, a group (without treatment), K(+) group as positive control, a group which were applied separator rubber and helical spring for orthodontic tooth movement, and P groups were applied with orthodontic force and treated with **S.hermanii** 3.5%. After treating the C. cobaya were sacrificed. Runx2 expression as a periodontal remodeling were examined with immunohistochemistry. The results showed that orthodontic tooth movement means and SD in K(-), K(+), P are 2.99±0.065; 4.38±0.035; 5.05±0.199; while the mean and SD of Runx2 expression is : 5. 25±1.031; 2.38±0.46; 12.13±0.875. Kruskal Wallis and Mann Whitney test exhibited orthodontic tooth movement and Runx2 expression were significantly increased with **S.hermanii** application (p<0,05). Based on the result we concluded that **S.hermanii** accelerate orthodontic tooth movement. Increasing Runx2 expression with **S.hermanii** showed that increasing periodontal remodeling.

**Keyword:** **S. hermanii**, Runx2 expression, orthodontic tooth

1. **Introduction**

Orthodontic treatment need a long time, approximately 6 month until 2 years. [1] Applying orthodontic force, caused reaction and remodeling of the periodontal tissue including alveolar bone. This is related to biological system as a result of mechanical force results in strain. [2] This force induce causing capillary vasodilatation within the blood vessels periodontal ligament, resulting in migration of inflammatory cells, releasing numerous neurotransmitter and cytokine, growth factors, colony-stimulating factors, and metabolites of arachidonic acid production resulting in remodeling and adaptation of the biological system to the newer circumstances. [3]

Orthodontic forces induce a complex bone remodeling response. Matrix metalloproteinases (MMPs) help in bone remodeling by breaking down the extracellular matrix. It has been found that, compression of periodontal ligaments induces an increase in MMP-1 levels 1 hour after mechanical loading. This increase lasted for 2 hours and subsequently disappeared. Tension within the PDL too resulted in significantly increased levels of MMP-1 protein after 1 hour of force application which also subsequently disappeared. [4] After that, bone cells have role in bone remodeling. Osteoclasts that responsible for bone resorption are mainly secreted from the macrophages and osteoblasts are produced by proliferations of the cells of the periodontal ligament. [2]

Runt-related transcription factor-2 (RUNX2) is a multifunctional transcription factor and is the first confirmed osteoblast specific transcription factor. It completed the regulation of skeletal development mainly by regulating the cell differentiation associated with the bone formation and the expression changes of extracellular matrix proteins. [5] In addition, RUNX2 is involved in the differentiation process of bone marrow mesenchymal stem cells into osteoblasts and play a key role in the mature process of osteoblast. [6]

Researchers now focus on finding methods to accelerate tooth movements. The best solution for shorting treatment time are to speed up the rate of tooth movement. There is some method to
accelerate orthodontic tooth movement. Stimulating orthodontic tooth movement through chemical agent such as parathyroid hormone, thyroxin, calcitonin, estrogen, Vitamins D3, osteocalcin and corticosteroid.[7] The other ways through physical agents such as vibration, heat, light, electric, magnetic fields and laser including low laser therapy. Accelerating orthodontic tooth movement also using corticotomy, piezoincision, microosteoperforation.[8] There is some evidence to suggest that low-level laser therapy and a corticotomy involving the raising of a muco-periosteal flap are associated with accelerated orthodontic tooth movement; however, the current level of evidence is low to moderate in quality.[7] No herbal agent used for accelerating tooth movement.

*S. hermanii* is well known as a human supplement nutritions. *S. hermanii* contain active ingredients such as proteins 86% (80% collagens), glucosaminoglycans such as hyaluronic acid, chondroitin sulphate, cell growth factor, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) that important for wound healing process. [9,10,11] It active content might be have role in accelerating orthodontic tooth movement. The aim of this study is to investigate *S. hermanii* stimulation to Runx2 expression in periodontal remodeling during orthodontic tooth movement.

### 2. Experimental Method

The study was 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 Hang Tuah University no 125/KEPK/I/2016. Twenty-four male guinea pigs (*C. cobaya*) aged 2.5 months and weighed 200-300 grams, fed with a standard pellet diet and tap water ad libitum, were randomly divided into three equal groups. The procedure of this study was started with acclimatization of animals for 48 hours. The Guinea pigs were divided into 3 groups. K(-) group is negative control group (without treatment), K(+) group is positive control group. The last group was triggered orthodontic tooth movement by using elastic separator, the force was 0.0474 kN and helical spring. P group were applied with both orthodontic forces and *S. hermanii* 3.5 % for 14 days. Choosing 3.5% *S. hermanii* based on previous research, the best *S. hermanii* concentration used were with 3.5%. *S. hermanii* gel 3.5% was made from 0.35 gr *S. hermanii* powder was diluted with NaCMC 2% in DMSO 5 % until 10 ml. *S. hermanii* gel was applied in gingival sulcus tension area 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 fourteen days of the experiment, anestesized with 10% ketamine injection as anesthetic drug were used, with 0.1-0.2 ml/kg for acepromazine 0.5 ml. The maxillary insisivi teeth were dissected and placed in 10% buffered formalin. Afterwards, histological section were prepared with Runx2 immunohistochemistry as osteoblast marker, and then observed by using a microscope. Observed the expression of Runx2 as osteoblast marker in the periodontal ligament on 1/3 apical in the tension area camera using optilab were taken to measure the Runx2 expression seen on the microscope with an enlargement 400x. Each histological section was observed and calculated.

Finally, the data were statistically measured by using Statistical Package for the Social Science (SPSS) version 20. The research data result tabulated, the statistical hypothesis was conducted with a standard analytic significance of 95 percent (p=0.05) by ANOVA test to analyze the difference of each variable compared with control. Then the data were tested with LSD Test (p<0.05).
3. Results and Discussion
The data of orthodontic tooth movement measurement showed that there were differences of orthodontic tooth movement width in each group. The measurement of orthodontic tooth movement was using caliper between distal incisive. The mean of negative control group of K (-), positive control group K(+) and P group were 2.99±0.065; 4.38±0.035 and 5.05±0.199, respectively.

Table 1. Descriptive mean and standard deviation of orthodontic tooth movement maxillary left central incisive (mm)

| Group | Orthodontic tooth movement (mm) | Mean | Standard Deviation |
|-------|---------------------------------|------|--------------------|
| K(-)  | 2.99                            | 0.065|                    |
| K(+)  | 4.38                            | 0.035|                    |
| P1    | 5.05                            | 0.199|                    |

Orthodontic force induce mechanical stress inducing reorganization of periodontal ligament (PDL) collagen bundles. These mechanism would result to orthodontic tooth movement. Mechanical stress transferred to the PDL causes tissue response causing tooth movement. This reaction of PDL is for the maintenance of homeostasis. [12] Data above showed that there are increasing orthodontic tooth movement that means increasing periodontal remodeling. The remodeling process occurring in periodontal tissues during orthodontic and orthopedic therapies may be a clinical usefulness procedure leading to proper choice of mechanical stress to improve and to shorten the period of treatment. [13]

Moreover, the data also showed that the Runx2 expression as osteoblast marker of increased in treatment groups. The highest number found in the P group treated with S. hermanii 3.5% is 12.13±0.875 cell/field of view. Meanwhile, in the negative control group K (-), the mean was 5.25±1.031 and in the positive control group K(+) the mean was 2.38±0.46 (figure 1). The statistical results of Kruskal Wallis and Mann Whitney test showed that Orthodontic tooth movement and Runx2 expression was significantly increased with administered S. hermanii application (p<0.05).

Figure 2. Histogram of mean and SD of Runx2 expression

S. hermanii contain various active ingredient such as hyaluronic acid, chondroitin sulphate, cell growth factor, EPA DHA, flavonoid. [10] In a previous in-vitro study exhibited that there was a positive promoting effect of S. hermanii water extract on osteoblast functional activity when 1.6mg/ml, 3.1mg/ml, 6.3mg/ml, 12.5mg/ml, and 25mg/ml of S. hermanii concentrations were used. Microscopic examination showed adequate cell merging in the wells with S. hermanii concentration from 1.6 mg/ml up to 25mg/ml. Previous studies indicated that the water extract of S. hermanii contains high amino acid concentrations (37%) as well as calcium, magnesium, iron and zinc that may play an important role in osteoblast molecular activities. [14]
The effect of glycosaminoglycan (GAG) such as chondroitin sulphate, oral administration had been shown to increase the total calcium pool and intestinal absorption of calcium, which may lead to an increased capacity for injured bone to regenerate during osteogenesis.[14] Condroitin sulphate on the surface of osteoblasts or bone matrix binds to cell adhesion molecule such as integrin on the pre-osteoclastic cells and inhibits the differentiation into osteoclasts so bone formation can occurred.[15]

Flavonoid, inhibits osteoclast differentiation and bone resorption in vitro but also stimulates human osteoblast differentiation. In vivo, flavonoid increases bone mass in immobilized rats and also the biomechanical properties of rat bone. [16] Flavonoid treatment resulted in a significant elevation of alkaline phosphatase (ALP) activity, collagen contents and osteoblast differentiation genes [ALP, collagen, osteopontin (OPN), osteoprotegerin (OPG) and osteocalcin (OC)] and bone morphogenetic protein (BMP) genes (BMP2, BMP4 and BMP7). [17] Flavonoid activated BMP signaling by inducing Smad1, phosphorylation, as well as Id1 and Id2 protein expression in a dose-dependent manner.[18]

Application of orthodontic force on periodontal tissue which influencing differentiation of periodontal ligament cells into osteoblasts to be involved in bone resorption and bone formation is the key of periodontal tissue remodeling of orthodontic tooth. [19] Orthodontic mechanical stimulation can induce periodontal ligament cells to differentiate into osteoblast in vitro. [20] Study the clinical application of tissue remodeling and mechanical signal transduction, which comprising many cytokines, extracellular matrix proteins and signal cellular. [21,22]

Studies in vitro found that runx2 competed an important regulation part in the differentiation of mesenchymal stem cells to osteoblast structure. [23] Baumert found that the expression of transcription factor correlated with the process of osteoblast differentiation and bone formation upregulated under the orthodontic pressure, which confirmed that these differentially expressed genes play a key role in mature process of osteoblasts terminal differentiation.[24]

The experimental results showed that the orthodontic force upregulated the expression of Runx2 the 14 days experiment period compared with the control group. On other hand, there was decreasing change in Runx2 expression in C. coba ya not subjected to S. hermanii application. These findings suggest that Runx2 may be involved in the early response of bone cells to mechanical signal. [25] In the osteoblast differentiation and bone formation, Runx2 as a marker for osteoblast and transcriptional factor with the runt domain can bind to the core regions in a number of enhancers and promoters.[26] Runx2 with other proteins in signaling pathways showed that they interact directly or indirectly with a number of molecules involved in bone remodeling, such as Run3, Sox9 and Msx2, forming a signaling network. [25]

4. Conclusion
Based on the result we concluded that S. hermanii accelerate orthodontic tooth movement. Increasing Runx2 expression with S. hermanii showed that increasing periodontal remodeling.

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