HANG TUAH UNIVERSITY

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INTERNATIONAL SCIENTIFIC MEETING

PROCEEDING BOOK

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Current Concepts and Technology in Improving Dental and Oral Health Care

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3rd DENTISPHERE (DENTISTRY UPDATE & SCIENTIFIC ATMOSPHERE) CURRENT CONCEPTS AND TECHNOLOGY IN IMPROVING DENTAL AND ORAL HEALTH CARE

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DEAN OF FACULTY DENTISTRY HANG TUAH UNIVERSITY WELCOME NOTE

Welcome to Surabaya!
Is a great honor for us to welcome you all at the International Seminar “Dentisphere 2016”. This international seminar is the third time we have held at the Shangri La Hotel Surabaya. This Seminar which held on 26-27 August 2016 is one of my pride as the Dean of Dentistry Faculty of Hang Tuah University. This is also proofing one of Hang Tuah University’s contribution both nationally and internationally in the field of dentistry.

The theme of International Seminar 3rd Dentisphere is "Current Concepts and Technology in Improving Dental and Oral Health Care", which aim is to provide a new generation of dentists who are experts and professionals with the knowledge that continues to grow for the Indonesian nation and the world. We hope that through this event we can raise the professionalism in the field of dentistry for all participants.

I would like to say a very big thanks to our speakers from home and abroad: Japan, Korea, Thailand, and Singapore. Thanks for all contributions and participation and your willingness to come and share your knowledge and experience in dentistry. It is an honor for us that the events will also have an important role in the quality control mechanisms to ensure stability and increased periodically in the field of dentistry.

Also for all the participants, thank you very much for joining the International Seminar 3rd Dentisphere, I hope you can all enjoy the entire summary of the seminar. Hopefully this seminar that we held useful for the advancement of knowledge of dentistry you all peers. I apologize if there are less pleasing for the organization of this seminar.

Enjoy the 3rd international seminar Dentisphere!

CHAIRMAN 3RD DENTISPHERE WELCOME NOTE

Hello Dentists!
Welcome to the International Seminar 3rd Dentisphere. It's an honor for us, Dentistry Faculty of Hang Tuah University to host the International Seminar 3rd Dentisphere. We are welcoming all of our sponsors, speakers and participants from both inside and outside Indonesia who contribute to this International event. Welcome to Surabaya!

The theme of this time seminar is "Current Concepts and Technology in Improving Dental and Oral Health Care", as the committee we offers a place to learn and exchange dental knowledge with national and international facilitators. International Seminar 3rd Dentisphere will also provide a unique opportunity for participants to develop the knowledge, skills and professionalism with the interaction with other participants. Do not miss the opportunity to interact directly and do hands on with the speakers and experts which are amazingly competent in the field of dentistry from different countries (Indonesia, Japan, Korea, Singapore, and Thailand).

After all, we apologize if if there are less pleasing for the organization of this seminar . Enjoy the beauty of the city of Surabaya while you also explore the dental sciences!

God bless us always.

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RESEARCH ARTICLE

Integrin A2β1 And Bmp-2 Regulated In Bone Remodelling To Accelerate Orthodontic Tooth Movement By Giving Stichopus Hermanii

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ABSTRACT

Background: *Orthodontic tooth movement is a continual and balanced process between bone deposition and bone resorption on pressure and tension sites. Integrin α2β1 is the major collagen type 1 receptor and BMP-2 is the parameter of osteoblast proliferation that have role in bone remodeling. Stichopus hermanii is one of the best fishery commodities in Indonesia, its contain various active ingredients such as hyaluronic acid,chondroitin sulphate,cell growth factor, EPA DHA, flavonoid that might have role in orthodontic tooth movement.* ***Objectives:*** *The aim of this study is to investigate Integrin α2β1 and BMP-2 regulated in bone remodelling to accelerate orthodontic tooth movement by giving Stichopus hermanii.* ***Material and Method:*** *Thirty two male Cavia Cobaya were divided into four groups. K(-) group as negative control group (without treatment), K(+) group as positive control group which were applied separator rubber for orthodontic tooth movement, and P1, P2 groups, were applied for orthodontic tooth movement and Stichopus hermanii 3 % and 3,5 %. After treatment the cavia cobaya were sacrificed. Integrin α2β1 and BMP2 expression were examined with immunohistochemistry.* ***Results:*** *This study showed Integrin α2β1 means and SD in K(-), K(+), P1,and P2 are 7,5±1,77; 3±1,07; 11,1±3,3 and 14,13±4,55. BMP-2 have means and SD : 5,38±2,72; 2,62±1,77; 10,88±3,64 and 18,63±1,5. Integrin was significantly increased in P2 and P1 compare to K(+), K(-), while BMP2 increased too.* ***Conclusion :*** *Stichopus hermanii active component could increase integrin α2β1 and BMP2 that regulate bone remodelling,while 3,5 % Stichopus hermanii had the best to accelerate orthodontic tooth movement.*

Keywords*: Stichopus hermanii, Integrin α2β1, BMP-2, orthodontic tooth movement.*

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BACKGROUND

Orthodontic tooth movement occurs in the presence of a mechanical stimuli sequenced by remodeling of the alveolar bone and periodontal ligament (PDL). Bone remodeling is a process of both bone resorption on the pressure site and bone formation on the tension site. Orthodontic tooth movement can be controlled by the size of the applied force and the biological responses from the PDL. The force applied on the teeth will cause changes in the microenvironment around the PDL due to alterations of blood flow, leading to the secretion of different inflammatory mediators such as cytokines, growth factors, neurotransmitters, colony-stimulating factors, and arachidonic acid metabolites. As a result of these secretions, remodeling of the bone occurs. [1,2]

Today, it is challenging to reduce the duration of orthodontic treatments. Long orthodontic treatment have potensial risk such as caries, gingival recession and root resorption. There are many ways to accelerate orthodontic tooth movement based on phases of tooth movement. There are three phases of tooth movement: the initial phase, which is characterized by rapid movement after the application of force; followed by a lag period, where little or no movement, and the last phase, where gradual or sudden increase of movement occurs. The early phase of tooth movement involves acute inflammatory responses characterized by leucocytes migrating out of blood capillaries and producing cytokines, which stimulates the excretion of prostaglandins and growth factors. The acute phase is followed by the chronic phase that involves the proliferation of fibroblast, endothelial cells, osteoblasts, and alveolar bone marrow cells remodeling process. [1, 2, 3]

High concentration of cytokines such as interleukins IL-1, IL-2, IL-3 IL-6, IL-8, and tumor necrosis factor alpha (TNF) were found to play a major role in bone remodeling; moreover, interleukin-1 (IL-1) stimulates osteoclast function through its receptor on osteoclasts. [4,5] Other cytokines which are also involved in the acceleration of tooth movement are RANKL, which is a membrane-bound protein on the osteoblasts that bind to the RANK on the osteoclasts and causes osteoclastogenesis.[6] Prostaglandins (PGs) are inflammatory mediator and a paracrine hormone that acts on nearby cells; it stimulates bone resorption by increasing directly the number of osteoclasts. *In vivo* and *in vitro* experiments were conducted to show clearly the relation between PGs, applied forces, and the acceleration of tooth movement. [7] Another set of investigators has made an experiment where they have injected vitamin D metabolite on the PDL of cats for several weeks; it was found that vitamin D had accelerated tooth movement at 60% more than the control group due to the increasement of osteoclasts on the pressure site.[8] Integrin α2β1 and BMP-2 regulate bone remodelling in last phase / chronic phase.

Many but there is no natural has been used for accelerating orthodontic tooth movement. Stichopus hermanii is one of the best fishery commodities in Indonesia. It is natural and contain various active ingredient such as collagen, hyaluronic acid, chondroitin sulphate,cell growth factor, EPA DHA, flavonoid that has been proved as [9] that might reduce relapse

orthodontic. Previous research showed that stichopus hermanii stimulated the activation and proliferation of fibroblasts, and enhanced rapid production of collagen fiber network with shorter healing time. The level of proinflammatory cytokines; IL-1α, IL-1β, and IL-6, were significantly reduced in Stichopus hermanii treated wounds and stimulation tissue regeneration. [10] Stichopus hermanii at 5 mg/ml and 10 mg/ml can increased osteoblast cell function. 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. [11] So, in this study, we investigate Integrin α2β1 and BMP-2 regulated in bone remodelling to accelerate orthodontic tooth movement by giving Stichopus hermanii

MATERIAL AND METHOD:

This study was performed on 32 male Cavia Cobaya 2,5 months old with 200-300 g weight. The Wistar rats was divided into 4 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 P1, P2, were applied with relaps orthodontic forces and Stichopus hermanii 2,5 % and 3 %.

Preparation of orthodontic tooth movement

Orthodontic forces was applied with giving applied separator by separating plier in mesial left insisivus maxilla cavia cobaya 14 days to produce orthodontic tooth movement. Separator forces was 0,0474 kN, measured by autograph

Preparation of Powder Stichopus Hermanii

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. Sticopus hermanii was dried but not be in direct sunlight for 7 days. After this, Stichopus hermanii was blender until get the powder.

Preparation and Applied Stichopus Hermanii gel

Stichopus hermanii gel 2,5% was made from 0,25 gr Stichopus hermanii powder was diluted with NaCMC 2% in DMSO 5 % until 10 ml. 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 once per day

The research was conducted in Biochemistry Laboratory Medical Faculty of Airlangga University. After 14 days of treatment. the cavia cobaya were sacrificed. The jaw was sectioned. Integrin α2β1 and BMP-2 (Bone Morphogenetic Protein-2) expression were examined with immunohistochemistry method in tension side.

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

RESULTS

The aim of this study is to investigate Integrin α2β1 and BMP-2 regulated in bone remodelling to accelerate orthodontic tooth movement by giving Stichopus hermanii. The result in this experiment show the the expression of integrin α2β1 in accelerating orthodontic tooth movement as shown as table 1

Table 1 : Integrin α2β1 in accelerating orthodontic tooth movement applied with Stichopus hermanii

Group	Mean± Standart Deviation
K(-)	7,5±1,77
K(+)	3±1,07
P1	11,1±3,3
P2	14,13±4,55

Table 1 show means and SD in K(-), K(+), P1,and P2 are 7,5±1,77; 3±1,07; 11,1±3,3 and 14,13±4,55.Then the data were tested with normality test, homogenity test and show the data was homogen and have a normal distribution. ANOVA test (p=0.05) for the expression of integrin α2β1 in accelerating orthodontic tooth movement applied with Stichopus hermanii showed significantly differences. With the LSD test, showed that integrin α2β1 expression : P1 and P2 showed increased integrin α2β1 expression whether P2 has the best expression as seen as table 2

Table 2 : LSD Test expression in relaps orthodontics Cavia Cobaya applied with Stichopus hermanii

Group	**K(-)**	**K(+)**	**P1**	**P2**
K(-)		0,006*	0,022*	0,000*
K(+)	0,006*		0,000*	0,000*
P1	0,022*	0,000*		0,055
P2	0,000*	0,000*	0,055	

*Significantly different

So, the expression of Integrin α2β1 was significantly increased in P2 and P1 compare to K(+) and K(-).

The result showed BMP-2 expresion were increased in accelerating orthodontic tooth movement by giving Stichopus hermanii as sees as table 3

Table 3 : The Expression BMP-2 as osteoblast activity in accelerating orthodontics tooth movement applied with Stichopus hermanii

Group	Mean± Standart Deviation
K(-)	5,38±2,72
K(+)	2,62±1,77
P1	10,88±3,64
P2	18,63±1,5

Table 3 show means and SD in K(-), K(+), P1,and P2 are 5,38±2,72; 2,62±1,77; 10,88±3,64 and 18,63±1,5 Then the data were tested with normality test, homogenity test and show the data was homogen and have a normal distribution. ANOVA test (p=0.05) for the expression of BMP-2 as osteoblast activity in accelerating orthodontic tooth movement applied with Stichopus hermanii showed significantly differences. With the LSD test, showed that BMP-2 expression : P1 and P2 showed increased BMP-2 expression whether P2 has the best expression as seen as table 4

Table 4 : LSD Test expression BMP-2 as osteoblast activity in accelerating orthodontics tooth movement applied with Stichopus hermanii

Group	K(-)	K(+)	P1	P2
K(-)		0,004*	0,000*	0,000*
K(+)	0,004*		0,000*	0,000*
P1	0,000*	0,000*		0,000*
P2	0,000*	0,000*	0,000*	

*Significantly different

So, the expression of BMP-2 was significantly increased in P2 compare to K(+), K(-) and P1.

DISCUSSION

The aim to this study was to investigate integrin α2β1 and BMP-2 regulated in bone remodelling to accelerate orthodontic tooth movement by giving Stichopus hermanii. This study showed the result Integrin α2β1expression means and SD in K(-), K(+), P1,and P2 were 7,5±1,77; 3±1,07; 11,1±3,3 and 14,13±4,55. BMP-2 had means and SD : 5,38±2,72; 2,62±1,77; 10,88±3,64 and 18,63±1,5. Integrin was significantly increased in P2 and P1 compare to K(+), K(-), while BMP2 increased too.

Orthodontic tooth movement in cavia cobaya models occurs when separator rubber applying in the left first insisivus compressed towards the distal side during 14 days orthodontic tooth movement. Increasing integrin and BMP-2 expresion by applying Stichopus hermanii during orthodontic tooth movement means there are processes for bone remodeling because integrin and BMP-2 plays a central role for alveolar bone osteogenesis.

Integrins are cell surface receptors composed of α- and β-subunits. Integrins enable cell adhesion (cell-matrix, cell-cell) and transduce both chemical and mechanical signals. Certain integrins have function to mediate mechanical stress-induced proliferation, shear stress activated extracellular regulated-protein kinases (ERKs) and c-Jun kinases (JNKs) and integrin may function as mechanotranduction.[12] A2β1 integrin is the major collagen type 1 receptor expressed on Th 17 cells that mediates attachment of collagen type 1.[13] α2β1 integrin increases collagen type 1 synthesis and turnover.[14]

The bone morphogenetic proteins (BMPs) included BMP-2, is the second family of growth factors, unique: these are the growth factors involved in the process of osteoblast differentiation that drive the process of bone formation and mineralization. Since the late 1980s, BMPs have been known to stimulate new bone formation. BMPs represent molecular targets used to identify and develop new agents to simulate the bone-forming process. Much is understood about the signal transduction pathway for the BMPs. BMP-2 stimulates the differentiation of mesenchymal cells into osteoblasts and chondrocytes. BMP-2 binds to its receptor, a Ser/Thr kinase, which phosphorylates and activates the intracellular signaling molecules Smad 1 and Smad 5. This in turn leads to the expression of the transcription factor Cbfa1 (Runx2), which results in the expression of several proteins critical for bone formation. Wnt/LRP5 pathway is also linked to the BMP pathway by a cascade of anabolic transcriptional events. The signal starts at the Hedgehog signaling pathway, moving through the BMPs and Wnt/LRP5, and ultimately leads to expression of the critical genes involved in osteoblast differentiation. This pathway provides multiple potential molecular targets that may be manipulated in the process

of bone formation. [15] The process that been needed to accelerated orthodontic tooth movement.

Stichopus hermanii contain various active ingredient such as collagen, hyaluronic acid, chondroitin sulphate,cell growth factor, EPA DHA, flavonoid.[9] In a previous in-vitro study showed that there was a positive promoting effect of stichopus hermanii water extract on osteoblast functional activity when 1.6mg/ml, 3.1mg/ml, 6.3mg/ml, 12.5mg/ml, and 25mg/ml of stichopus hermanii concentrations were used. Microscopic examination showed adequate cell confluency in the wells with stichopus hermanii concentration from 1.6 mg/ml up to 25mg/ml. Previous studies showed that the water extract of Stichopus contains high amino acid concentrations (37%)34 as well as calcium, magnesium, iron and zinc that may play an important role in osteoblast molecular activities. [11]

Previous study showed that increasing integrin α2β1 mediates cell adhesion to and spreading on fibrillary collagen. Integrin α2β1 also can mediate collagen gel contraction and promote the integrin-mediated formation of long cellular projections typically that has role in mechanical tension. Condroitin Sulphate on the surface of bone matrix binds to cell adhesion molecule such as integrin. Ascorbic acid is also can promote collagen integrin.[16] Collagen type 1 is a major type for matrix composition in alveolar bone formation of orthodontic tooth movement.

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.[15,17] 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). [18] Flavonoid activated BMP signaling by inducing Smad1, 5 phosphorylation, as well as Id1 and Id2 protein expression in a dose-dependent manner. [19]

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.[11] 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. [16]

Stichopus hermanii accelerate tooth movement through integrin α2β1 and BMP-2 in bone remodelling cycle. Stichopus hermanii Bone remodeling process is a last phase in orthodontic tooth movement that occur after rapid movement stops. When tooth movement occurs, bone resorption have role in bone remodeling. Bone formation is a phase after bone resorption. Increasing integrin α2β1 and BMP2 regulate bone formation process. Bone formation process increasing so that bone remodelling cycle.

CONCLUSION

Stichopus hermanii active component could increase integrin α2β1 and BMP2 that regulate bone remodelling,while 3,5 % Stichopus hermanii had the best to accelerate bone remodelling in orthodontic tooth movement.

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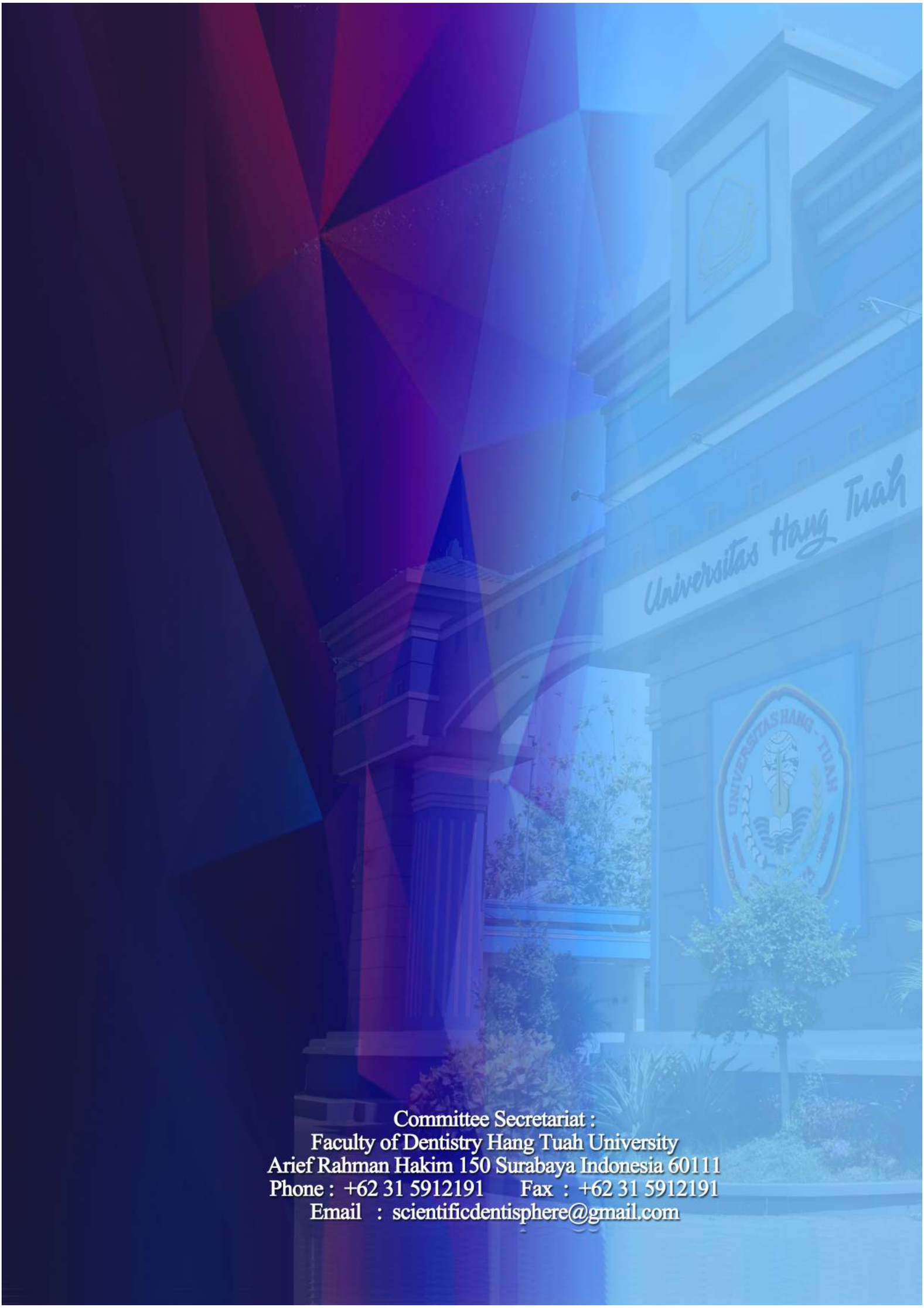

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