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EVALUATION OF ALVEOLAR BONE MICROMORPHOLOGICAL CHANGES IN EXPERIMENTALLY INDUCED PERIODONTAL DISEASE RAT MODEL BY USING MICRO-COMPUTED TOMOGRAPHY (MICRO-CT)

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It is hereby certified that we have read this project paper entitled "Evaluation of Alveolar Bone Micromorphological Changes in Experimentally Induced Periodontal Disease Rat Model by Using Micro-Computed Tomography (Micro-CT)", by Chew Xuan-Yee and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirement for the course VPD 4999–Final Year Project.

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DEDICATIONS

FAMILY

To Giek King and Wee Chin for their unconditional love

And Mianzi, Mianxin and Yan-Yu for their continuous support

LECTURERS

To my supervisor and co-supervisor for all their guidance, advices and assistance

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To my beloved classmates and rotamates of batch 2013/2018

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LIST OF ABBREVIATIONS

LPS	**Lipopolysaccharide**
***Pg*-LPS**	***Prophyromonas gingivalis* Lipopolysaccharide**
Micro-CT	**Micro Computed Tomography**
BV/TV	**Percent Bone Volume**
TbTh	**Trabecular Thickness**
BMD	**Bone Mineral Density**
PoTot	**Total Porosity**
Pg/Fn	***Prophyromonas gingivalis/Fusobacterium nucleatum***
VOI	**Volume of Interest**
ROI	**Region of Interest**
p-Akt	**Protein Kinase B**
TLR4	**Toll-like Receptor 4**
NF-κB	**Nuclear Factor-κappa B**
IL-1β	**Interleukin1β**
IL-6	**Interleukin 6**
IL-8	**Interleukin 8**
RANK	**Receptor Activator of NF-κB Ligand**
BALB/c	**An Albino, Laboratory-bred Strain of House Mouse**
ANOVA	**Analysis of Variance**

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999–Projek Ilmiah Tahun Akhir

PENILAIAN PERUBAHAN MIKROMORFOLOGI TULANG ALVEOLAR PADA MODEL TIKUS YANG DIJANGKITI PENYAKIT PERIODONTAL SECARA EKSPERIMEN DENGAN PENGGUNAANTOMOGRAFI TERKOMPUTERAN MIKRO (MICRO-CT)

oleh

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2018

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Penyelia bersama: Dr. Lau Seng Fong, Dr. Rozanaliza Radzi

Periodontitis didorong oleh disbiosis bakteria periodontopatogenik yang boleh mengakibatkan kehilangan tulang alveolar. Tujuan kajian ini adalah untuk menilaiperubahan mikromorfologi tulang alveolar yang disebabkan oleh ligatur, suntikan LPS dan gabungan suntikan LPS dalam model tikus. Data imbasan mikro-CT dari 72 hemimaxilla tikus dianalisis menggunakan Skyscan 1076. Kumpulan rawatan terdiri daripada Kawalan, Ligatur, Ligatur-lipopolisakarida (Ligature-LPS) dan *Porphyromonas gingivalis*-lipopolisakarida (*Pg*-LPS). Setiap rawatan terdiri daripada 7, 14 dan 30 hari.Pada hari 7, kumpulan Ligatur and Ligatur-LPS menunjukkan penurunan ketara ($p < 0.05$) dalam jumlah tulang

peratus (BV / TV), ketebalan trabekular (TbTh), dan kepadatan mineral tulang (BMD), manakala jumlah porositi (PoTot) meningkat, menunjukkan penyerapan tulang. Perubahan ini secara beransur-ansur berkurang, dan pada hari ke 30, mereka tidak signifikan ($p > 0.05$) berbanding dengan kumpulan Kawalan. Ini mungkin menunjukkan kejadian pembentukan semula tulang. Kumpulan *Pg*-LPS tidak menunjukkan perubahan ketara dalam semua parameter. Keputusan ini menunjukkan bahawa Ligatur and Ligatur-LPS menyebabkan penyerapan tulang lebih banyak daripada *Pg*-LPS dalam tikus.

Kata kunci: Penyakit periodontal, model Ligatur, model Ligatur-lipopolisakarida (Ligature-LPS), *Porphyromonas gingivalis*-lipopolisakarida(*Pg*-LPS), parameter tulang.

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfillment of the course VPD 4999 Final Year Project.

EVALUATION OF ALVEOLAR BONE MICROMORPHOLOGICAL CHANGES IN EXPERIMENTALLY INDUCED PERIODONTAL DISEASE RAT MODEL BY USING MICRO-COMPUTED TOMOGRAPHY (MICRO-CT)

by

Chew Xuan-Yee

2018

Supervisor: Assoc. Prof. Dr. Chen Hui Cheng

Co-supervisors: Dr. Lau Seng Fong, Dr. Rozanaliza Radzi

Periodontitis is driven by dysbiotic of periodontopathogenic bacteria that may lead to alveolar bone loss.The purpose of this study was to evaluate the alveolar bone micromorphological changes inducedby ligature, injection of LPS and combination of ligature-LPS injection in the rat model.Micro-CT scanned data from 72 hemimaxilla of previously induced rats were analysed using Skyscan 1076. Treatment groups consisted of Control, Ligature, Ligature-lipopolysaccharide (Ligature-LPS) and *Porphyromonas gingivalis*-lipopolysaccharide (*Pg*-LPS). Eachtreatment comprised of day 7, 14 and 30. At day 7, both Ligature and Ligature-LPS groups showed significant decrease ($p<0.05$) in percent bone volume (BV/TV), trabecular thickness (TbTh), andbone

mineral density (BMD), but increased intotal porosity (PoTot),indicating bone resorption. These changes gradually reduced, and byday 30, they were insignificant ($p>0.05$) when compared to the Control group. This likely indicate the occurrence of bone remodeling. The*Pg*-LPS group showed no significant change in all parameters. These results showed that Ligature and Ligature-LPS caused more bone resorption than *Pg*-LPS in rats.

Keywords: Periodontal disease, Ligature model, Ligature-lipopolysaccharide (Ligature-LPS) model, *Porphyromonas gingivalis*-lipopolysaccharide (*Pg*-LPS), bone parameters

1.0 INTRODUCTION

Periodontitis is defined as a chronic inflammatory disease affecting tooth supporting tissues, initiated by a synergistic and dysbiotic microbial community bacteria as the primary etiologic factor and culminating in the destruction of the dental attachment apparatus (Hajishengallis & Lamont, 2012). Host defense, genetic and environmental factors are responsible for different periodontal conditions that can be observed in humans (Libermanl et al., 2011). While gingivitis does not affect the underlying supporting structures of the teeth, periodontitis results in loss of connective tissue and bone support and can progress to bone destruction, tooth mobility and finally, tooth loss (Martins et al., 2016).

Various animal species were used in experimental study models in periodontology, for instance, rats, hamsters, rabbits, ferrets, dogs, pigs and primates. All of these animals were used in order to mimic human periodontal diseases in an attempt to reveal pathogenetic mechanisms of periodontal disease and find cure (Balci Yuce, 2016). Rodents, with rats in particular, are important models for experimental periodontal research because rats are easy to handle and inexpensive (Oz & Puleo et al., 2011). One of the most successful study approaches, germ-free or gnotobiotic rats such as Sprague-Dawley rats, made the study of dental plaque and bacterial biofilm in periodontal research possible.

Balci Yuce (2016) depicted that Ligation model and Lipopolysaccaride (LPS) injection model are few periodontal disease induction method that has been used in previous study. Ligation model with silk suture material is the most common way of inducing periodontal disease, however, other retentive device are acceptable to be used as well. The ligatures are thought to facilitate local accumulation of bacteria and thereby enhance bacteria-mediated inflammation and bone loss (Graves et al., 2008). Meanwhile for LPS, it has been suggested that LPS can penetrate gingival connective tissue and induce a local inflammatory response that leads to periodontal bone resorption (Cheng et al., 2010). However, to induce the same lesion, this method may need a longer time as it is associated with inducing chronic periodontal disease (Bostanci & Belibasakis, 2012). On the other hand, combination of Ligature and LPS has not been described clearly in previous studies.

Therefore, this study aims to evaluate the alveolar bone micromorphological changes induced by ligature, injection of LPS and combination of ligature-LPS injection in the Sprague Dawley rat model. The hypotheses were:

Ho: There is no significant difference in Percent Bone Volume (BV/TV), Trabecular Thickness (TbTh), Total Porosity (PoTot) and Bone Mineral Density (BMD) among Ligature, Ligature-LPS and Pg-LPS injection in periodontal disease induction.

Ha: There is significant difference in Percent Bone Volume (BV/TV), Trabecular Thickness (TbTh), Total Porosity (PoTot) and Bone Mineral Density (BMD) among Ligature, Ligature-LPS and Pg-LPS injection in periodontal disease induction.

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