

Research Report

## Differences Post-extraction Collagen-density of Wistar-rat with Aerobic and Anaerobic Interval-training

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### ABSTRACT

**Background:** Tooth extraction is extracting teeth from alveolar bone. In the world, tooth loss is a disease that affects the function and aesthetics oral cavity, it causes 7.5 million Disability-Adjusted Life Year (DALY). Tooth extraction rate in Indonesia is around 24% in 2013 and 19% in 2018. Sports is one of the activities that can help accelerate wound healing after tooth extraction, this study examine differences in collagen density after tooth extraction of Wistar rats given aerobic and anaerobic interval training. **Purpose:** To prove differences in post-extraction collagen density of Wistar Rat (*Rattus norvegicus*) with aerobic and anaerobic interval training. **Methods:** Wistar rats were divided into 3 groups, control group (K) rat were soaked in a bucket with tap water as high as the rat soles with a duration of 50% of maximum swimming ability (KRM), aerobic interval (P<sub>1</sub>) 3% load swim test with duration of 3 sets 50 % KRM exercise and 25% KRM rest, anaerobic interval P<sub>2</sub> load 6% duration 80% KRM exercise and rest with 4 sets. All groups were observed on the 3rd and 7th day post extraction. Data were analyzed using Kruskal-Wallis and continued with Post-hoc test to test differences between groups. **Results:** There was only significant difference in collagen thickness between groups K<sub>(3)</sub> and K<sub>(7)</sub> and another groups had not significant difference. **Conclusion:** there insignificant differences in collagen density after tooth extraction in Wistar rats (*Rattus norvegicus*) with aerobic and anaerobic interval training.

**Keywords:** aerobic exercise; anaerobic exercise; collagen; physical activity; tooth extraction

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### INTRODUCTION

Exodontia is a procedure of extracting teeth from the alveolar bone socket because the tooth destruction due to caries, trauma, and periodontal disease. In the world, tooth loss causes 7.5 million people suffered Disability-Adjusted Life Year (DALY).<sup>1</sup> Based on 2018 RISKESDAS, Indonesian tooth extraction rate is 19% and shows an increasing trend in the age group above 65 years.<sup>2</sup>

Under normal condition, post-extraction wounds go through 4 stages of healing such as hemostasis, inflammation, proliferation, and remodeling.<sup>3</sup> If wound healing phase is disrupted, it will cause complications and one of them dry socket.<sup>4,5</sup> Sports is a physiological activity to improve the body's fitness status and body health,<sup>5,6</sup> this activity has many advantage one of them is accelerating wound healing.<sup>7</sup>

Several studies have shown exercise can improve wound healing at every stage of wound healing. In hemostatic

phase, exercise increasing fibrinogen expression to enhance clot stabilization.<sup>8</sup> Exercise can reduce the expression of cytokines and proinflammatory chemokines, while it can reduce the overexpression of macrophages and polymorphonuclear leukocytes so that the inflammatory process does not last long.<sup>9</sup> Increased VEGF expression by type 2 macrophages and fibroblast synthesis also occur in wound healing given by exercise.<sup>10</sup>

Collagen is an extracellular matrix component that plays a role to maintain tissue strength and integrity, in wounds collagen has important role to facilitating granulation tissue formation, cell adhesion & migration, and cells interactions.<sup>11</sup> Based on previous research, acceleration of extraction wound healing in remodeling phase has never been studied, therefore researchers want to observe the effect of aerobic & anaerobic interval sports on collagen density after tooth extraction. The aim of this study was to prove the differences in collagen density after tooth extraction in Wistar rats (*Rattus norvegicus*) by aerobic and anaerobic interval training.

**MATERIALS AND METHODS**

The design of this study was pure experimental using Wistar rats as experimental animals. The study design uses a post-test only control group design. This research was accepted by the ethics committee of Airlangga University, No. 417 / HRECC.FODM / VI / 2019.

The subject was randomized and divided into three groups, namely; control group (K), aerobic interval exercise (P<sub>1</sub>), anaerobic interval exercise (P<sub>2</sub>). Then Wistar Rat was adapted for seven days and the body weight was measured. Before conducting a swim test, the measurement of Maximum Swimming Capacity (MSC) is needed to determine the swim test duration.

Each group was treated 3 times every week, for 6 weeks. The control group was not given any treatment and only soaked with duration 50% MSC. The aerobic interval training was given swim test with a load of 3% of body weight, the total duration 50% MSC with 3 sets of exercise and 2 sets of rest. Anaerobic interval training group carried out swim test duration of 80% MSC with a load of 6% of body weight, exercises carried out 4 sets of exercise and 3 sets of rest. On day 43, rats were anesthetized using intramuscular injection of ketamine 3.6 ml, after that left mandibular incisors were extracted and irrigated. Post days 3 & 7 of extraction the rat was euthanasia using ether and socket tissue was taken.

The tissue was taken for histopathological examination using Masson Trichrome staining. Furthermore, collagen density was observed by scoring method at 400x magnification. The results obtained were statistically analyzed using post-hoc Dunn-Bonferroni.

**Table 1.** Mean and standard deviation of collagen density.

Groups	Sample	Mean	Standard Deviation
K <sub>(3)</sub>	7	1.71	0.756
P <sub>1(3)</sub>	7	2.43	0.757
P <sub>2(3)</sub>	7	2	0.758
K <sub>(7)</sub>	7	2.71	0.759
P <sub>1(7)</sub>	7	3.43	0.760
P <sub>2(7)</sub>	7	2.86	0.761

**RESULTS**

On the study, 42 samples of wistar rats divided in 6 group namely; 2 control groups euthanized on day-3 (K<sub>3</sub>) and day-7 (K<sub>7</sub>); 2 interval aerobic groups euthanized on day-3 (P<sub>1(3)</sub>) and day-7 (P<sub>1(7)</sub>); 2 interval anaerobic groups euthanized on day-3 (P<sub>2(3)</sub>) and day-7(P<sub>2(7)</sub>). Table 1. it showed mean and standard deviation of collagen density scoring, the table showed that P<sub>1(7)</sub> has the highest mean of collagen density scoring. Table 2. it showed different test post hoc Dunn-Bonferroni with signification p<0.05, based on the table only K<sub>3</sub> and K<sub>7</sub> showed significance difference with p= 0.049, the other groups did not show significance difference with p>0.05.

**DISCUSSION**

Collagen is one of extracellular matrix components that acts as the foundation of extracellular matrix (scaffold), it synthesized by fibroblasts in the proliferation phase, that scaffold facilitates cells morphogenesis and tissue regeneration.<sup>12,13</sup> Descriptive analysis of the results of the study based on Table 1. The average score of aerobic exercise both on day 3 and day 7 showed the highest results when compared with other treatment groups on the same day. In the control group showed the lowest scoring results compared to all groups both treatment day 3 and 7.

Post hoc analytic test to conduct different tests in each group Table 2. In group K<sub>(3)</sub> and K<sub>(7)</sub>, the result of p is 0.049 the p value shows a significant difference (p<0.05). On day 3 is the initial stage of formation of collagen which is stimulated by fibroblasts during the proliferation phase, and collagen deposition continues to rebuild the integrity & strength of the tissue. Compared to the 3rd day, collagen formation on the 7th day has better results because on the 3rd day there is a transitional phase between the inflammatory process and proliferation so that the formation of collagen has not occurred maximally.<sup>14</sup>

On the 3rd day there were insignificant results in each group, the results of p were K<sub>(3)</sub> & P<sub>1(3)</sub> (0.136), K<sub>(3)</sub> & P<sub>2(3)</sub> (0.566), P<sub>1(3)</sub> & P<sub>2(3)</sub> (0.359) with significance levels (p<0.05). Insignificant results obtained due to overlapping between the inflammatory phase with the proliferation phase so that the formation of collagen has not occurred

**Table 2.** Post-hoc Dunn-Bonferroni significance test

Groups	K <sub>(3)</sub>	P <sub>1(3)</sub>	P <sub>2(3)</sub>	K <sub>(7)</sub>	P <sub>1(7)</sub>	P <sub>2(7)</sub>
K <sub>(3)</sub>		0.136	0.566	0.049*		
P <sub>1(3)</sub>	0.136		0.359		0.054	
P <sub>2(3)</sub>	0.566	0.359				0.081
K <sub>(7)</sub>	0.049*				0.149	0.731
P <sub>1(7)</sub>		0.054		0.149		0.271
P <sub>2(7)</sub>			0.081	0.731	0.271	

Note: Black tables are not post hoc tested.  
 (\*) indicates significant difference (p<0.05).

optimally.<sup>14</sup> Day 7 also found insignificant results in all comparisons in each group with the results *p* is greater than the difference level (0.05). Insignificant results occur because on MT staining all types of collagen and it will turn blue, at the beginning of healing the wound type III collagen is formed and fill the wound area, after that type I collagen replaces type III collagen by metalloproteinase released by fibroblasts and macrophages, The aims is to improve the stability of the extracellular matrix.<sup>14,15</sup> The process of degradation of type-III collagen to type-I affects the results obtained because in MT all collagen will colored blue and cannot be specifically differentiated.

The insignificant results obtained could be due to the subjective scoring system, the distance between scoring which is not uniform so that the standardization of collagen scoring is needed. Investigations are also needed such as checking blood lactic acid levels to distinguish aerobic and anaerobic exercise.<sup>16</sup> Besides supporting examinations, one of which is the measurement of hydroxyproline levels, metalloproteinase matrix, and expression of growth factors is also needed to determine whether exercise can affect collagen density after extraction.<sup>17</sup> Based on the discussion above it can be concluded that aerobic and anaerobic interval exercise not affect on post extraction wound collagen density .

## ACKNOWLEDGEMENT

Gratitude and appreciations are addressed to all staffs of the Research Center - Faculty of Dental Medicine Airlangga University, and the Laboratory of Biochemistry - Faculty of Medicine Airlangga University

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