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Universidad del Zulia
Facultad Experimental de Ciencias
Departamento de Ciencias Humanas
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TGF-β1 Expression in Osphronemus gouramy Scale Application in Rattus novergicus Tooth Socket

Chiquita Prahasanti[1]
[1]Staff in Departement of Periodontology, Indonesia
chiquita-p-s@fkg.unair.ac.id

Anneke Paramita Adityatama[2]
[2]Resident in Periodontology Specialist Program, Faculty of Dental Medicine, Universitas Airlangga, Surabaya - Indonesia
anneke-p-a@fkg.unair.ac.id

Okkinardo Arief[3]
[3]Resident in Periodontology Specialist Program, Faculty of Dental Medicine, Universitas Airlangga, Surabaya - Indonesia
okkinardo-a@fkg.unair.ac.id

Onge Victoria Hendro[4]
[4]Resident in Periodontology Specialist Program, Faculty of Dental Medicine, Universitas Airlangga, Surabaya - Indonesia
onge-v-h@fkg.unair.ac.id

Abstract

The aim of the study is to observe an enhancement of TGF-β1 expression in gourami fish-scale application in Wistar rat tooth socket. As a method, thirty-six experimental animals were randomly divided into 4 groups as 7 and 14 days' control group and 7 and 14 days' fish-scale group. TGF-β1 expression was analyzed under immunohistochemistry on days 7 and 14. Results: Statistic test one-way ANOVA with a significant number 0.000 ($p < 0,05$) and Tukey HSD multiple comparisons showed a significant difference between groups. In conclusion**,** a Fish-scale scaffold derived from gourami can enhance the expression of TGF-β1.

Keywords: Gouramy, Fish-scale, Scaffold, TGF- β1.

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La expresión TGF-β1 en la aplicación de la escala de gouramy de Osphronemus en el diente de la rata parda

Resumen

El objetivo del estudio es observar una mejora de la expresión de TGF-β1 en la aplicación de escamas de pescado gourami en el alveolo de rata Wistar. Como método, treinta y seis animales experimentales se dividieron aleatoriamente en 4 grupos como grupo de control de 7 y 14 días y grupo de escamas de pescado de 7 y 14 días. La expresión de TGF-β1 se analizó bajo inmunohistoquímica en los días 7 y 14. Resultados: La prueba estadística ANOVA unidireccional con un número significativo 0.000 ($p < 0,05$) y las comparaciones múltiples de Tukey HSD mostraron una diferencia significativa entre los grupos. En conclusión, un andamio a escala de pescado derivado de gourami puede mejorar la expresión de TGF-β1.

Palabras clave: Gouramy, Escama de pescado, Andamio, TGF-β1.

1. INTRODUCTION

As chronic inflammation disease, periodontitis can cause the destruction of tooth-supporting tissue. Alveolar bone breakdown induces tooth to lose in the mouth (RUSYANTI, WIDYAPUTRA & MELANI, 2019). To restore bone destruction in periodontitis, the dentist can regenerate defective tissue. Tissue reconstruction success depends on a few components as a good progenitor cell, appropriate signaling of the molecule, and there are blood supply and scaffold in defective tissue.

A bone graft can improve success in periodontal tissue regeneration and new bone formation through osteogenesis, osteoinductive and osteoconductive (HUANG, 2016). The bone graft must have a few criteria as safety in application, biocompatible, and bioactive (osteogenesis, osteoinductive and osteoconductive). Osteoinductive and osteoconductive are the most important reason that the dentist must use a biomaterial because this material can accelerate tissue formation (KHAN & KHAN, 2013).

As biomaterial, collagen has many advantages like biodegradation, biocompatible, easy to be obtained, and other benefits. Collagen is an extracellular matrix component (ECM) as a protein in the body of life things (HUANG, 2016). As a bone graft, collagen has many usabilities in dentistry, because collagen has many advantages as good adhesion in the wound, hemostatic agent, binding a tissue fluid and vascular stimulation. Ability to enhance wound healing through clot formation and neovascularization.

Fish scale as collagen basic material is a biocompatible material. In vitro cytotoxic study exhibit fish scale not induce a significant cytotoxic effect and this material has enough cell viability. Experiment by PRAHASANTI, WULANDARI & ULFA (2018), collagen extract from the gourami fish scale is type 1 collagen (PRAHASANTI ET AL, 2018).

The healing process is starting with blood clot formation, revascularization and growth factor existence. This process is to be

marked with the existence of transforming growth factor-β (TGF-β) superfamily. TGF-β superfamily can induce many mesenchymal cells to differentiate into osteoblast and pluripotent stem cells as chondrocyte germ. The tallest synthesis and response found in bone, thrombocyte, and cartilage. TGF-β1 is the widest isoform in protein and cytokine that has many functions in physiologic activity including the tissue healing process. The healing process of the bone is happened by TGF-β1 through regulation, differentiation, and activation osteoblast and osteoclast (ESTAI, 2011). Many studies indicate TGF-β1 has an important role in cell growth, homeostasis, disease, and recovery of the wound. Because of that, the experiment to see the expression of TGF-β1 in the bone healing process in fish scale application is very important.

2. MATERIAL AND METHODS

The fresh fish scale was collected from the market washed with flowing cool water and then the fish scale was save in refrigerator with -25^{o}C temperature until its used. The fish scale was soaked in 6% acetat acid fluid for 7 days and acetat acid fluid changed every day. During the soaked process, the sample was kept in a refrigerator with 4^{o}C temperature. The result of fish scale immersion was float and the pH neutralized by flowing water with pH indicator paper. At the time when neutral pH was achieved, collagen fiber was seen. The collagen clot was perfected in neutral pH (pH 7). After that, the collagen clot

was filtered with filtered paper and its dried with the freeze-drying method.

This experiment has been through an ethics commission with number #216/KKEPK.FKG/IX/216. This experiment used 36 healthy male Wistar rats with 3 months' age and 150-300-gram weight, the rats were divided into 2 observation groups in 7 and 14 days randomly. The 7-day group divided into a control group. In the control group, incisivus mandibula was extracted and the socket left until the blood fills the socket. The treatment group was given gourami fish scale in socket incisivus mandibula. The observation did on the fourteenth day. TGF-β1 expression was observed in both groups.

The trial animal was anesthetized with ketamine (1,04 mg/kg) intramuscular, left mandibular insisivus was extracted and then socket was irrigated with saline. In the first group, the socket left until the blood fill the socket (control group). In the second group, the socket filled with a gourami fish scale and then the socket was sutured. In the seventh and fourteenth days, the trial animal was sacrificed with 10% ether inhalation anesthetized. After the animal stopped breathing, the incisivus mandibula Wistar rat was taken and its fixated in 10% buffer formalin for prevented the change of tissue structure. After 48 hours, the fixation fluid changed with the new fluid and tissue cut off into small pieces so fixation fluid can penetrate equally into the tissue. In this second stage, tissue left into the fluid for 48 hours. After fixation, tissue was soaked into 10% EDTA decalcification fluid for 1 month and fluid changed every day.

Expression of TGF-β1 was observed quantitatively by immunohistochemistry technique with a light microscope by 400 x magnification and the result calculated using tool image and then recorded. This technique for determining antigen location (protein target) in tissue or cells with antigen-antibody reaction. Observation TGF-β1 expression in osteoblast cell did with routine coloring technique and continued with the immunohistochemistry technique.

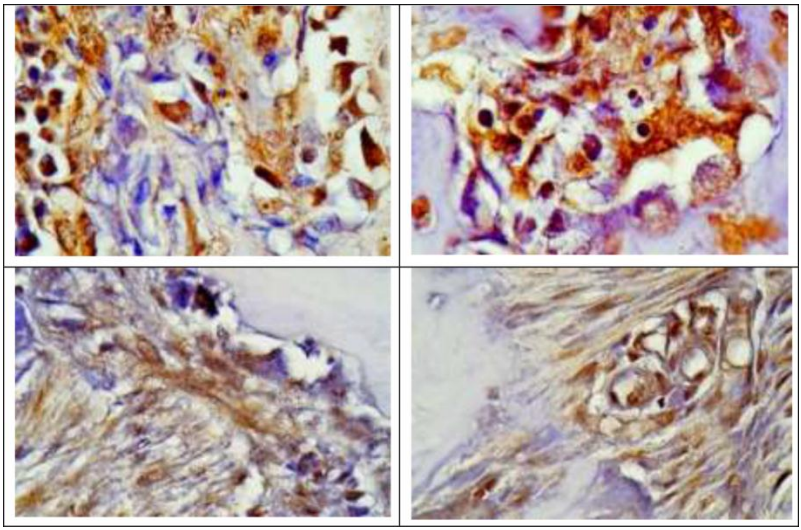

Figure 1: Histologic image from TGF-β1 expression (1000x magnification)

3. RESULT

The quantitative calculation of TGF-β1 expression based on the intensity of color in each group with an OLYMPUS BX microscope

series 41 with DP-71 digital camera. This is histological features under microscopic observation:

Table 1: *One-way* ANOVA test result

	Sum of squares	Degree of freedom	The mean squared	F	Sig.
Between group	308.972	3	102.991	33.030	0.000
Within group	99.778	32	3.118		
Total	408.750	35			

Data analysis by one-way ANOVA (Analysis of Variance) showed 0.00 significance ($p < 0.05$), which means there were significant differences for each control group and treatment group with the gourami fish scales scaffold.

4. DISCUSSION

Collagen is a natural biomaterial, acts as a scaffold and its have osteoinductive and osteoconductive properties, which means it is a favored spot for the cell penetration and formation of new bone. After collagen crosslinked into collagen fibrils, collagen will mature into collagen fiber which will provide a place for deposition and growth of hydroxyapatite crystals. PRAHASANTI ET AL (2018) did SEM analysis to see morphology and porosity of the collagen extract from gourami fish scales (PRAHASANTI ET AL, 2018). Morphology of

collagen extracted from gourami fish scales looked like fibers interconnected (meshwork) with pores among them, similar to collagen extracted from freshwater fish observed by (PATI ET AL., 2012).

The results showed the expression of TGF-β1 highest in fish scale group on day 14th, there was a significant difference in TGF-β1 expression between fish scale group on day7th compared with the control group on day 7th, there is a significant difference between fish scale group on day 14th and control group on day 14th. The osteogenic response started on day 7th until day 25th, when bone remodeling and bone maturation occurs on the 14th day until the 35th day, because of that measurement TGF-β1 expression did on day 7th and day 14th. This situation showed that osteogenic response began on day 7th when remodeling and bone maturation occurs on the 14th day. On the 7th day, TGF-β1 began to increase and continued until day 14th. It shows that TGF-β1 played a role in the early stages of osteoblast differentiation and it is same with KASAGI & CHEN (2013), that TGF-β1 increased the production of extracellular matrix proteins of bone in the early stages of osteoblast differentiation that is at preosteoblasts formation and the formation of immature osteoblasts.

The fish scales collagen groups showed expression of TGF-β1 is higher than the control group, these results indicated the application of fish scale collagen as a scaffold can increase the expression of TGF-β1 in osteoblasts. Control group on day 14th compared with fish scale group day 7th showed no significant difference. It showed fish scale

collagen as a scaffold will accelerate osteoblast differentiation. The application of gouramy fish scales collagen not only increasing the expression of TGF-β1 but also accelerate osteoblast differentiation. Interaction between cell-matrix and osteoblasts to bone matrix proteins including type I collagen is important for osteoblast function and differentiation (NAKAMURA, 2007).

5. CONCLUSION

Three-dimensional structure of gourami fish scale collagen resembles the shape of ECM bone components, which serves to facilitate the growth of vascularization into the scaffold and to provide an ideal environment for bone formation (KAJIMURA, 2013). The pore size of the graft that used for bone tissue engineering purposes must have pore sizes ranging between 200-900 μm from SEM test that already conducted by PRAHASANTI ET AL (2018) showed the pore size of the collagen extract of gourami fish scales ranged from 191.6 to 385.3 μm, its same as the pore size required as a scaffold in tissue engineering (PRAHASANTI ET AL, 2018). The development of collagen material as a material for hard tissue engineering is necessary to add other mineral materials in the bone such as calcium phosphate or crosslinking with materials such as hydroxyapatite (PARENTEAU-BAREIL, GAUVIN & BERTHOD, 2010).

Increased expression of TGF-β1 showed that applied collagen has the ability to stimulate bone formation because TGF-β1 will

stimulate proliferation and osteoblast mineralization, this condition indicated that collagen can increase bone proliferation (BOSETTI, 2007). Based on this research, it can be concluded that the application of gourami fish scales (Osphronemus gouramy) collagen as a scaffold on tooth extraction sockets in Wistar rats (Rattus norvegicus) can increase the expression of TGF-β1 as bone maker proliferation.

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