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**BIOCOMPABILITY TEST OF HARUAN FISH (*Channa striata*) BONE
 HYDROXYAPATITE TO FIBROBLAST CELL AS PERIODONTAL POCKET
 THERAPY**

*(In Vitro Study on BHK-21 Fibroblast Cell with Hydroxyapatite of Haruan Fish Bone (*Channa striata*) as Bone Graft Material)*

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ABSTRACT

Background: Periodontitis is an inflammation of the soft tissues and hard tissues that support the tooth characterized by periodontal pocket formation, recession to resorption of alveolar bone. So far, alveolar bone resorption caused by periodontitis can be treated with bone graft therapy. Xenograft is a type of bone graft that has many advantages such as can be obtained from natural materials, low in price and has minimal virus transmission. Hydroxyapatite of haruan fish bone is a type of xenograft material that has never been applied in medical field. **Objective:** This study aims to analyze the biocompatibility of haruan fish bone hydroxyapatite (*Channa striata*) against BHK-21 fibroblast cells via viability. **Method:** This was a laboratory experimental study with posttest only control group design, using MTT assay method and BHK-21 fibroblast cell viability was calculated using optical density formula. **Results:** The concentrations of 0,2109 mg/ml, 0,4218 mg/ml 0,8437 mg/ml, 1,6875 mg/ml, 3,3375 mg/ml and 6,75 mg/ml were biocompatible against fibroblast cells BHK-21 whereas at concentrations of 54 mg/ml, 27 mg/ml and 13,5 mg/ml were toxic to BHK-21 fibroblast cells. One Way Anova test and Bonferroni test showed concentrations of 54 mg/ml, 27 mg/ml and 13,5 mg/ml had significant differences to other concentrations. **Conclusion:** Hydroxyapatite of haruan fish bone (*Channa striata*) at some concentration is biocompatible against BHK-21 fibroblast cells.

Keywords: *Channa striata*, bone graft, hydroxyapatite, biocompatibility.

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INTRODUCTION

Periodontitis is an inflammation of soft tissue and hard tissue that support the tooth, characterized by periodontal pocket formation, gingival recession and alveolar bone resorption that reduce the support of the tooth.^{1,2} Poor oral health and hygiene are contributing factors of periodontitis.^{3,4} So far, the resorption of alveolar bone caused by periodontitis can be treated with bone graft therapy.^{5,6,7}

Bone graft therapy is a therapy to replace damaged bone tissue with a certain material that can be obtained from the patient's own body, synthetic materials or natural materials.^{8,9} A graft material must have the ideal characteristics for the recipient, which are non-toxic, does not cause infection, easy to adapt and can stimulate new attachment.¹⁰

There are various type of graft material, one of them is *xenograft*. The advantages of *xenograft* are it can be obtained from natural materials, available

in large quantities, does not require surgery procedure for the retrieval of materials and minimal virus transmission.^{11,12} The disadvantage of *xenografts* is the high price, which leads the interest of many researchers to look for alternative material that is affordable and has good quality for periodontal pocket therapy.

Haruan fish bone is one of raw materials that can be used for *xenograft*, because it has organic and inorganic components.¹³ Haruan fish is common in South Kalimantan especially Banjarmasin, so the haruan fish bone is very easy to find. People in Banjarmasin believe haruan fish can be used as a medicine to accelerate post operation wound healing process post-operation because it contains high protein, amino acids, fatty acids and minerals.^{13,15,16,17}

Calcium contained in haruan fish bone plays a role in the formation of crystals apatite during bone formation.^{18,19} Hydroxyapatite (HAp)(Ca₁₀(PO₄)₆(OH)₂) in haruan fish bone is the major component or mineral that chemically and physically similar to human's teeth and bone.^{20,7} Before being applied to human, the hydroxyapatite of haruan fish bone needs to pass through a biocompatibility test so it does not cause any adverse reactions.²¹ The purpose of this study is to analyze the biocompatibility of hydroxyapatite of haruan fish bone on BHK-21 fibroblast cells.

MATERIAL AND METHOD

This research began by getting research permit and ethical clearance issued by Faculty of Dentistry, Lambung Mangkurat No. 063/KEPKG-FKGULM/EC/IX/2017. This research used laboratory experimental with *posttest only control group design*. The samples used were the hydroxyapatite of haruan fish bone obtained from the Laboratory of Jaringan Biologi Badan Tenaga Nuklir (BATAN) Jakarta and BHK-21 (Baby Hamster Kidney) fibroblast cells obtained from the Laboratory of Peningkatan Mutu Pengembangan Produk (PMPP) Pusat Veteriner Farma (PUSVETMA) Surabaya.

The total sample was obtained using *Lemeshow* formula, which was 2 samples for each treatment group. This study consisted of 11 treatment groups, which were 9 groups of haruan fish (*Channa*

striata) hydroxyapatite with various concentrations of: 54 mg/ml, 27 mg/ml, 13,5 mg/ml, 6,75 mg/ml, 3,375 mg/ml, 1,6875 mg/ml, 0,8437 mg/ml, 0,4218 mg/ml, 0,2109 mg/ml. One group of control cell as the positive control group with living percentage of 100% and one group of media control group medium that does not contain culture medium as the negative control group, with living cell percentage of 0%.

The Preparation of Hydroxyapatite of Haruan Fish Bone.

Haruan fish bone was obtained from a home-made crackers manufacture place. Preparation of the hydroxyapatite fish bone began with the washing and cleaning of the fish bones from the remnants of flesh and debris left behind. The fish bone was cut into chips shape using a bone-cutting machine, then the bones were washed again with high pressure water to remove the bone marrow and soft tissue on the bone surface. The fat in the bone was extracted with chloroform:methanol (1:1). The rest of the extracting agent was eliminated with the aquades, then the bone was crushed until the size of ± Mesh 60 was attained. The bone samples were de-proteinized by hydrolysis process using sodium hydroxide (NaOH) with concentration of 1 M at 70°C for 7 hours, then precipitated for 24 hours and filtered. The de-proteinized bone was dried with oven at 60 °C and filtered with 200 mesh (± 75 µm) or nm size. De-proteinized bone was packaged in screw-covered bottles and sterilized with gamma ray irradiation.

Biocompatibility Test on Hydroxyapatite of Haruan Fish Bone with MTT Assay Method

BHK-21 fibroblast cells, *eagle's* media and 10% FBS were cultured in roux bottles at 37°C incubators. Cell proliferation was done until the BHK-21 fibroblast cells attached to the wall of roux bottle. After it was filled, the *eagle's* and FBS media solutions were removed. The roux bottles were washed 3 times with PBS, then tripsine versene was added to release the cell from the bottle wall and the cell release process was stopped with the *eagle's* medium and FBS 10%. 80 well of BHK-21 fibroblast cells were transferred to a

microwell plate, and given hydroxyapatite of haruan fish bone according to the concentration. Incubation was done \pm 24 hours using CO₂ incubator, then washed 3 times with PBS. MTT reagent was added and re-incubated for 2-4 hours. Then, the MTT solution was discarded and added DMSO. Microwell plate was shaken for 5 minutes and cell viability was read using *ELISA reader* with 620 nm wavelength. The percentage of living cells was calculated using *Optical Density* formula^{14,22}:

$$\% \text{ cell alive} = \frac{(\text{OD treatment} + \text{OD media}) \times 100\%}{(\text{OD cell control} + \text{OD media})}$$

Note:

- % cell alive: percentage of living cell after testing
- OD treatment: *Optical Density* value on each treatment sample.
- OD media: *Optical Density* value on media control.
- OD cell control: *Optical Density* value on control cell.

Based on calculation with formula above, a material is stated not toxic if it has \geq 60% of living cell.

RESULT

Based on observation using visual MTT staining and calculation with *Optical Density* formula on hydroxyapatite of haruan fish bone with concentration of 0,2109 mg/ml, 0,4218 mg/ml, 0,8437 mg/ml, 1,6875 mg/ml, 3,375 mg/ml and 6,75 mg/ml were not have toxic to BHK-21 fibroblast cells, while concentrations of 13,5 mg/ml, 27 mg/ml and 54 mg/ml were toxic to BHK-21 fibroblast cells (Figs. 1 and table 1). The result of this study stated that the lower the concentration showed higher viability of fibroblast cells BHK-21 (Figure 2).

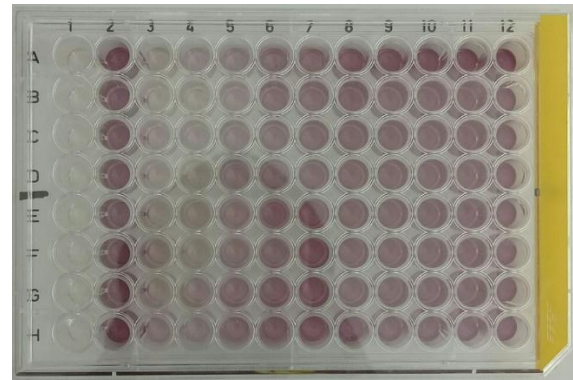


Figure 1. *Microwell Plate*96 wellcontaining BHK-21 fibroblast cell after given hydroxyapatite of haruan fish bone.

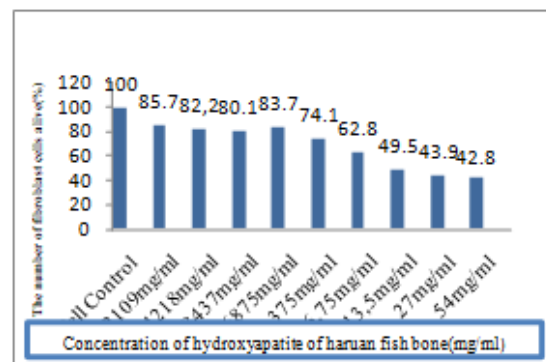


Figure 2. Graph of average value of living fibroblast cell on all treatment groups.

Table 1. Mean and standard deviation of the total number of living BHK-21 fibroblast cells on each group.

Group	Mean	Standard Deviation
Control Cell	100.0000	0.00000
0,2109 mg/ml	85.7500	3.04056
0,4218 mg/ml	82.2000	2,68701
0,8437 mg/ml	80.1000	4.94975
1,6875 mg/ml	83.7500	3.74767
3,375 mg/ml	74.1000	0.56569
6,75 mg/ml	62.8000	3.95980
13,5 mg/ml	49.5500	0.21213
27 mg/ml	43.9500	2.19203
54 mg/ml	42.8500	2.05061

The result of the research data were analyzed using *Shapiro-Wilk* normality test and *Levene's Test* for homogeneity test which resulted in the data distributed normally and homogenous, then continued with *One Way Anova* test. The result was $p = 0,000$ ($p < 0, 05$) which showed that there was significant difference between the average of cells percentage in all groups. The test was continued with a *Post Hoc Bonferroni* test to determine which groups had significant difference in the cell percentages. The result showed that at concentrations of 54 mg/ml, 27 mg/ml and 13,5 mg/ml had a significant difference to other concentrations and between the three there were no significant differences, where all three concentrations were toxic.

DISCUSSION

The result of the toxicity test of hydroxyapatite from haruan fish bone with MTT assay method based on visual observation on the color difference in each concentration and the value of *Optical Density* in each concentration showed that the higher the concentration the lower the viability of BHK-21 fibroblast cells. The lowest viability was found in hydroxyapatite of haruan fish bone with concentrations of 54 mg/ml, 27

mg/ml and 13,5 mg/ml. This is because the these concentrations create more viscous solvent than other concentrations, which differentiate the ability of the fibroblast cell membrane and inhibit the proliferation of BHK-21 fibroblast cells.²²

The decreasing viability of BHK-21 fibroblast cells at high concentrations can also be caused of the decreasing in ATP levels. ATP is the energy required for cell activity and produced by dehydrogenase enzymes. The toxic effect from the hydroxyapatite of haruan fish bone at high concentrations lead to inactive dehydrogenase enzyme so the required ATP for the cell decreases and causes the death of BHK-21 fibroblasts cell.²²

Sjerobabin *et al* (2016) stated that in a toxicity test, the higher the hydroxyapatite concentration the lower viability of mouse fibroblast cells found. The result of this research is established by the increasing concentration and the cell morphology change which was influenced by the length of contact time between the culturing medium and the material. Also, the decreasing of viability can occur because of the decrease in pH level, while the normal pH used for cell growth is about 7-7,4.^{23,24} According to Kamal *et al* (2013) on toxicity tests, the solvents and the materials needs to be adjusted as the cellular environment so they do not inhibit the proliferation of cell.²⁵

Based on a study by Manoj *et al* (2015) on increasing concentrations, the nanostructure of hydroxyapatite can increases particle size, which cause low crystallinity and damage to the cell membranes.²⁶ Based on Thirivikraman *et al* (2014) nanostructure of hydroxyapatite has higher solubility and can increase the release of calcium and phosphorus ions. The nanostructure of Hydroxyapatite at concentration of ≥ 4 mg/ml may cause damage to DNA and induce apoptosis reaction.²⁷

The highest viability for the BHK-21 fibroblast cells from the hydroxyapatite of haruan fish bone found in concentration 0,2109 mg/ml, then followed by 1,6875 mg/ml, 0,4218 mg/ml, 0,8437 mg/ml, 3,375 mg/ml and 6,75 mg/ml. The reason is because at these concentration, the BHK-21 fibroblasts are more adaptable which create the solvent to be easier to dissolve in the media and in the media culture.^{23,28}

According to Chung *et al* (2014) the crystals size and porosity are highly influence the biological environment during cell attachment, tissue growth, nutrient transfer and cell proliferation.²⁹ Thomas and Gupta (2017) suggest that calcium and phosphorus contents in hydroxyapatite create adaptable environment for fibroblast cells, and so this is what increases the viability of fibroblast cells.³⁰

So far hydroxyapatite is a material that has been widely used in treating periodontal defects. According to Mondal *et al* (2012), hydroxyapatite from fish bone is a natural inorganic material that is biocompatible to healthy living tissues. According to Mustafa *et al* (2015), hydroxyapatite from natural ingredients such as fish bones has metabolic activity and give good response compared to synthetic hydroxyapatite.¹⁶ According to Rismanchian *et al* (2013), nano size hydroxyapatite has good bioactivity, because it has larger surface area.²⁸

Based on research by Saleh *et al* (2014), nano sized hydroxyapatite proved to be able to increase the proliferation and maturation of fibroblast cells, which lead to regeneration of connective tissue during healing process of damaged periodontal.³² Based on research by Kamboj *et al* (2016), nano sized hydroxyapatite is capable of reducing pocket depth and increase the attachment significantly.³³

Based on the results of this study it can be concluded that the hydroxyapatite from haruan fish bone at some concentrations are biocompatible to BHK-21 fibroblast cells. The concentrations were 0,2109 mg/ml, 0,4218 mg/ml, 0,8437 mg/ml, 1,6875 mg/ml, 3,375 mg/ml and 6,75 mg/ml.

REFERENCES

1. Newman, Takei, Klokkevoold, Carranza. *Clinical Periodontology*. 12th ed. Canada: Julie Eddy; 2015. p. 50-2.
2. Tanna NK, Marjorie K, Jeffcoat. Actonel Risedronate Therapy for the Maintenance of Alveolar Bone in Adult Chronic Periodontitis. 2013;5(7):12-17.
3. Rustam A, Tatengkeng F, Fahrudin AM, Djas AI. Kombinasi Perancah Silk-Fibroin dari Kepompong Ulat Sutera Bombyx Mori dan Konsentrat Platelet Sebagai Inovasi Terapi Regenerasi Tulang Alveolar. 2017; 6 (3): 107-115.
4. Fadhilah N, Irhamni, Jalil Z. Sintesis Hidroksiapatit yang Berasal dari Tulang Sapi Aceh. *Journal Of Aceh Phisics Society (JAcPS)*. 2016; 5 (2): 19-21.
5. Hienz SA, Paliwal S, Ivanovski S. *Mechanisms of Bone Resorption in Periodontitis*. Hindawi Publishing Corporation. 2015; 1 (15): 1-10.
6. Cahaya C, Masulili SL. *Perkembangan Terkini Membran Guided Tissue Regeneration*. 2015;1 (1):1-11.
7. Ardhiyanto HB. Stimulasi Osteoblas Oleh Hidroksiapatit Sebagai Material Bone Graft Pada Proses Penyembuhan Tulang Stomatognatic (J.K.G Unej). 2012; 9 (3): 162-4.
8. Mao T, KamakshiV. Bone Graft and Bone Substitutes. *International Journal of Pharmacy and Pharmaceutical Sciences*. 2013; 6 (2): 88-91.
9. Dewi PS. Penatalaksanaan Kerusakan Tulang Pasca Pencabutan dengan Teknik Bone Grafting. 2012; 2 (5): 1-5.
10. Kandwal A, Bhardwaj J, Sunny, Batra M. Bone Graft in Periodontal Surgery. *Journal of Dental Herald*. 2014;1 (3): 030-2.
11. Singh J, Takhar RK, Bhatia A, Goel A. Bone Gaft Materials: Dental Aspects. *International Journal of Novel Research in Healhcare and Nursing*. 2016; 3 (1): 99-103.
12. Jamjoom A, Cohen R E. Graft for Ridge Preservation. *Journal of Functional Biomaterials*. 2015; 6 (3): 833-48.
13. Cucikonada Y, Supriadi A, PurwantoB. Pengaruh Perbedaan Suhu Perebusan dan Konsentras NaOH Terhadap Kualitas Bubuk Tulang Ikan Gabus (*Channa striata*). 2012; 1 (1): 91-101.
14. Apriasari ML, Adhani R, Savitri D. Uji Sitotoksisitas Ekstrak Metanol Batang Pisang Mauli (*Musa sp*) Terhadap Sel Fibroblas BHK Baby HamsterKidney 21. *J Dentino*. 2014; 2 (2): 210-14.
15. Asfar M, Tawali AB, Mahendradatta M. SNTI-B13 Potensi Ikan Gabus (*Channa striata*) Sebagai Sumber Makanan Kesehatan-Review. *Seminar Nasional Teknologi Industri II*. ISBN: 978-602-14822-1-6; 2014. p. 150-4.
16. Mustafa N, Ibrahim M, Asmawi R. Hidroxyapatite Extracted From Waste Fish Bones and Scales via Calcination Method. 2015; 773 (774): 287-290.

17. Agustin R, Dewi N, Rahardja SD. Efektivitas Ekstrak Ikan Haruan (*Channa striata*) dan Ibu Profen Terhadap Jumlah Sel Neutrofil Pada Proses Penyembuhan Luka. *J Dentino*. 2016; 1 (1): 68-74.
18. Tangalayuk RR, Suarsana IN, Utama IH. Kadar Kalsium dan Fosfor Pada Tulang Tikus Betina yang Diberi Tepung Tempe Rendah Lemak. *Buletin Veteriner Udayana*. 2015; 1 (7): 59-65.
19. Tua B, Amri A, Zultiniar. Sintesis dan Karakterisasi Hidroksiapatit dari Cangkang Kerang Darah dengan Proses Hidrotermal Variasi Suhu dan PH. *Jom FTEKNIK*. 2016; 3 (2): 1-5.
20. Fitriawan M, Amalia SR, Saputra BA, Setyawati E, Yulianto A, Mahardika. Sintesis Hidroksiapatit Berbahan Dasar Tulang Sapi dengan Metode Pretipitasi sebagai Kandidat Pengganti Graft Berdasarkan Compressive Strength. *Seminar Nasional Fisika*. ISBN 978-602-1034-071-1. 1 ed; 2014. p. 318-22.
21. Anusavice KJ. *Buku Ajar Ilmu Bahan Kedokteran Gigi*. 10th ed. Jakarta: EGC; 2014. p. 62-64
22. Emilda Y, Budipramana E, Kuntari. Uji Toksisitas Ekstrak Bawang Putih (*Allium sativum*) Terhadap Kultur Sel Fibroblas. *Dental Journal*. 2014; 47 (4): 215-19.
23. Sjerobabin N, Colovic B, Petrovic M. Cytotoxicity Investigation of a New Hydroxyapatite Scaffold With Improved Structural Design. 2016; 1445 (5-6): 280-87.
24. Bintarti TW, Izak RD, Ady J. Sintesis dan Karakterisasi *Bone Graft* Berbasis Hidroksiapatit dan Alginat. 2013; 2 (1): 108-25.
25. Kamal AF, Iskandriati D, Dilogo IH. Biocompatibility of Various Hydroxyapatite Scaffolds Evaluated By Proliferation of Rat's Bone Marrow Mesencymal Stem Cell: an In Vitro Study. *Medical Journal of Indonesia*. 2013; 22 (4): 202-8.
26. Manoj M, Subbiah R, Manglaraj D. Influence of Growth Parameters on the Formation of Hydroxyapatite (HAp) Nanostructures and Their Cell Viability Studies. *Nanobiomedicine*. 2016; 2 (2): 1-11.
27. Thrivikraman G, Madras G, Basu B. In Vitro/ In Vivo Assessment and Mechanisms of Toxicity of Bioceramic Materials and its Wear Particulates. 2014; 1 (4): 12763-81.
28. Rismanchian M, Khodaeian N, Bahramian L. In Vitro Comparison of Cytotoxicity of Two Bioactive Glasses in Micropowder and Nanopowder Forms. *Iranian Journal of Pharmaceutical Research: IJPR*. 2013; 12 (3): 437-43.
29. Chung JH, Kim YK, Kim KH. Synthesis, Characterization, Biocompatibility of Hydroxyapatite Natural Polymers Nanocomposites For Dentistry Application. 2014; 44 (1): 277-84.
30. Thomas B, Gupta K. In Vitro Biocompatibility of Hydroxyapatite added GIC: an SEM Study Using Human Periodontal Ligament Fibroblast. *J Esthet Restor Dent*. 2017; 29 (6): 435-41.
31. Mondal S, Mondal B, Dey A. Studies On Processing and Characterization Of Hydroxyapatite Biomaterials From Different Bio Wastes. *JMMC*. 2012; 11 (1): 55-67.
32. Saleh RG, El Tokhey AO, El Guindi HM. Evaluation of Hydroxyapatite Nanoparticles With and Without Silver Nano-Particles in The Treatment Of Induced Periodontitis In Dogs. *J AM Sci*. 2014; 10 (12): 21-23.
33. Kamboj M, Harinder RA, Gupta, Comparative Evaluation Of The Efficacy Of Synthetic Microcrystalline Hydroxyapatite Bone Graft (Ostim) and Synthetic Microcrystalline Hydroxyapatite Bone Graft (Osteogen) In The Treatment Of Human Periodontal Intra-bony Defect. *J Indian Soc Periodontal*. 2016; 20 (4): 423-8.