Viability Test of Collagen Extract from Gouramy Scale (*Oshpronemusgouramy) on Bone Marrow Mesenchymal Stem Cell Culture*

Chiquita Prahasanti[1], Widya Rizqi Romadhona[2], Shafira Kurnia S.[2], Aristika Dinaryanti[3]

[1]Professor in Department of Periodontics Dental Medicine, Faculty of Dental Medicine, Universitas Airlangga, Surabaya Indonesia, [2]*Dental Medicine Professional Program, Faculty of Dental Medicine, Universitas Airlangga, Surabaya Indonesia,* [3]*Research Center for Stem Cell Research and Development Institute of Tropical Disease, Universitas Airlangga, Surabaya Indonesia*

Abstract

Background: The role of type I collagen is as a matrix of extracellular proteins with characteristics of increased cell proliferation which directly affects the physiology and morphology of cells. Type 1 collagen can be obtained from fish scales. Thus, this research aimed to support engineering tissue used for the treatment of periodontal disease in the regenerative surgery by utilizing collagen derived from gouramy scales. As an initial step, the researchers wanted to conduct a study used collagen extract derived from gouramy scales (Osphoronemusgouramy) which was applied to bone marrow mesenchymal stem cell cultures to see viability in vitro.

Objective: To determine the viability of collagen from fish scales (Osphronemusgouramy) to bone marrow mesenchymal stem cells. Method: Bone marrow mesenchymal stem cells are taken from mice and planted in 96 well plates. Collagen extracted from gouramy scales using the enzymatic method was dissolved in a condition medium with each concentration of 0.01 mg/ml, 0.02 mg/ml, 0.04 mg/ml, 0.16 mg/ml, 0.32 mg/ml was put into the well prepared and incubated for 24 hours for the MTT assay.

Result: Collagen from fish scales can increase the viability of bone marrow mesenchymal stem cells with a percentage above 90% and the highest viability concentration at 0,01 mg/ml. Conclusion: Collagen from fish scales is viable against bone marrow mesenchymal stem cells. Collagen scales of gouramy soaked in medium had the highest viability with an optimum dose of 0.01 mg/ml.

Keywords: *Bone marrow mesenchymal stem cell, gouramy scales collagen, viability.*

Introduction

The regenerative material used in the field of dentistry has grown rapidly now. Regeneration of periodontal tissue with regenerative materials is expected to work at the cellular level. Used of regenerative materials can trigger cell proliferation and differentiation that are useful for the development of various cells.

Type I collagen is as a matrix of extracellular proteins capable of increasing cell proliferation. Type 1 collagen can be obtained from fish scales. Fish scales contain components including 70% water, 27% protein, 1% fat, and 2% ash. Organic compounds consist of 40% -90% in fish scales and the rest are collagen.[2-4]

Gouramy (*Osphronemusgoramy*) is one of the aquaculture products whose production increases every year[5]. The amount of gouramy consumption reached

Correspondence Author:
Chiquita Prahasanti
Professor in Department of Periodontics, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia
Contact No.: +62816531471
e-mail: chiquita_prahasanti@yahoo.com
chiquitaprahasantiunair@gmail.com

33.89 kg/capita/year in 2012 and in 2013 it increased to 35.14 kg/capita/year.[6] Zhang's 2011 study showed collagen derived from freshwater fish scales was safer to use than collagen sourced from other materials such as cattle and pigs and collagen derived from freshwater fish scales containing high type 1 collagen.[7] Hence, this research aimed to the development scaffold to support tissue engineering to treat periodontal disease in the regenerative field by utilizing collagen derived from carp scales. As an initial step, the researchers wanted to conduct a study using collagen extract derived from gouramy scales (*Osphoronemusgouramy*) which was applied to bone marrow mesenchymal stem cell cultures to see viability in vitro.

Materials and Method

The extraction of gouramy fish scales into collagen was conducted at the Instrumental Analysis Laboratory of the State Polytechnic Chemical Engineering Department, Malang. Culture of bone marrow mesenchymal stem cell and viability tests conducted at the Stem Cell Institute of Tropical Disease Research and Development Center, Surabaya.

Collagen Extracted from Gouramy Fish Scales: Gouramy scales were washed with water and dried under the sun to dry. After that, soaked in 1 M NaOH solution, 4°C for 24 hours to remove non collagen protein. NaOH solution was replaced every 8 hours and occasionally stirring. Washing with distilled water was performed to neutralize the pH followed by immersion in isobutanol to remove fat and addedchelating agent (EDTA) 1N for decalcification. Followed by the addition of acetic acid (acid solubility collagen) as much as 0.5 M and the addition of the enzyme pepsin (pepsin solubility collagen) as much as 0.1 gr, then stirred with an ultrasonic device at 4 º C. Then filtering, then added NaCl 0, 5 M, and centrifugation in small tubes with a speed of 4000 rpm, after that washed with distilled water and then disposed (salting out). The lyophilization process with a freeze dryer to remove the water content with a condenser temperature of -76 º C and ambient temperature of 23.6 º C for 12 hours until the water runs out. The results of collagen extract of carp scales are sterilized.

Preparation of Conditioned Medium: Collagen extract of gouramy scales was soaked in α-MEM medium for 24 hours.

MTT Assay Test on Bone Marrow Mesenchymal Stem Cell: Samples were calculated by using the Federer formula with 5 treatments, 1 cell control, 1 medium control, and 4 replications were obtained. The concentrations used in this study were 0.32 mg/ml, 0.16 mg/ml, 0.04 mg/ml, 0.02 mg/ml and 0.01 mg/ml. Microplate containing cells was incubated for 24 hours in a 5% CO_2 incubator at 37ºC. Microplates were removed from the incubator, culture media and scales of gouramy were removed then cell cultures in well washed with PBS. In each well, 100 µL of MTT solution was added. Cells were incubated for 4 hours in a 5% CO_2 incubator at 37ºC. After the incubation period was complete, discard the medium and sample. Then stop by giving DMSO to each well. The value of formazan optical density was read by using ELISA reader with a wavelength of 590 nm. The live cell percentage was calculated and the CD50 price was analyzed by SPSS. The reading results were converted into% by using the formula to determine the percentage of cell viability:

$$\% \text{ of live cells} = \frac{\text{Treatment + Media x 100\%}}{\text{Cell + Media}}$$

Note:

% of live cells = Percentage of live cell count after the test

Treatment = Optical density value of formazan in each sample after the test

Media = Optical density value of formazan on media

Cell = Optical density value of formazan on control cells.

Results

Table 1. Optical Density in the treatment group of gouramy scales collagen which was dissolved in the medium of conditions.

Group	N	Mean OD ± SD	Viability ± SD (%)
Control Cell	4	0,787 ± 0,033451	100 ± 0,00642
0,01 mg/ml	4	0,902 ± 0,008386	116,502 ± 0,06263
0,02 mg/ml	4	0,882 ± 0,051046	113.632 ± 0,07160
0,04 mg/ml	4	0,858 ± 0,043432	110.188 ± 0,06837
0,16 mg/ml	4	0,837 ± 0,151168	107.175 ± 0,24156
0,32 mg/ml	4	0,739 ± 0,047455	93,184 ± 0,05863

The percentage of living cells reflects the value of cell viability in the sample. The increase in the number

of bone marrow mesenchymal stem cells was evidenced by the comparison of the average life percentage of the control sample group which is 100%.

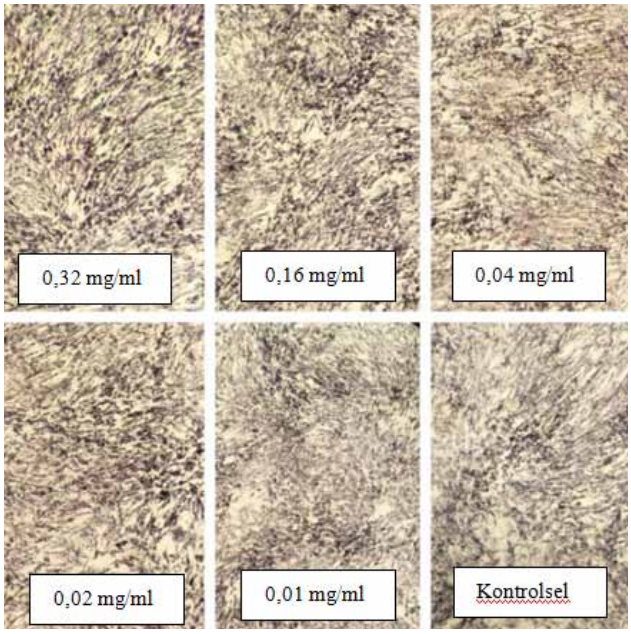
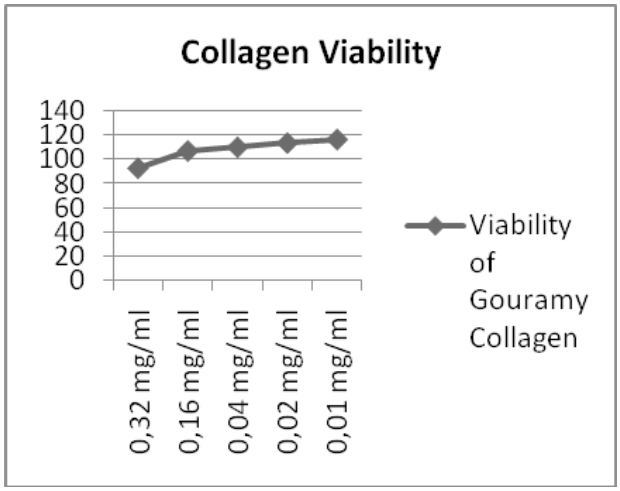


Figure 1. Description of a microscope bone marrow mesenchymal stem cell after being treated

The average percentage of living cells in all concentrations was above 100% except at a concentration of 0.32 mg/ml. From the results table of the percentage calculation of living cells can be graphed as follows:

Figure 2. Graph of percentage of living cells



Statistical Analysis:

The normality test is used the Kolmogrof-Smirnov Test. The basis of decision making in this test is:

1. If the value of Sig. > 0.05, then the data is normally distributed
2. If the value of Sig. <0.05, then the data is not normally distributed

Table 2. The results of the normality test of the collagen extract treatment group soaked in the medium.

Kolmogrof-Smirnov Statistic	Sig.
0,289	0,000

The following are the results of the normality test of each treatment group of collagen extract soaked in a medium that shows all groups have Sig. amounting to 0.000 which is less than 0.05 (p <0.05) which means that all groups are not normally distributed.

Table 3. Test results of the homogeneity of the treatment group of collagen extract soaked in the medium

Levene Statistic	Sig.
4.789	0.003

Then a variance homogeneity test was conducted with Levene's Test in the treatment group of collagen extract soaked in the medium obtained p = 0.003[8]. This shows that this data is not homogeneous because it does not meet p> 0.05.

Data is not normal and not homogeneous, then it is followed by the Kruskal-Wallis significance test.

Table 4. Kruskal-Wallis test results

Group	P value	Alfa
Collagen	0,005	0,05

P value was lower than the alpha significance level in all treatment groups, so there was a significant difference in concentration of one with the other concentrations.

Data analysis is continued by using Mann-Whitney Test to compare differences between concentration groups. Mann-Whitney Test statistical tests showed that there were significant differences between each medium control group with a concentration of 0.32 mg/ml, 0.16 mg/ml, 0.04 mg/ml, 0.02 mg/ml, 0, 01 mg/ml. Significant differences were also found in cell control and concentration of 0.04 mg/ml, cell control with a concentration of 0.01 mg/ml, concentration of 0.32

mg/ml with a concentration of 0.04 mg/ml, 0.02 mg/ml, 0.01 mg/ml. While the cell control group with 0.32 mg/ml, the cell control group with a concentration of 0.16 mg/ml, the cell control group with a concentration of 0.02 mg/ml, a concentration of 0.16 mg/ml with a concentration of 0.04 mg/ml, 0.02 mg/ml, 0.01 mg/ml, concentration of 0.04 mg/ml with a concentration of 0.02 mg/ml and 0.01 mg/ml, concentration of 0.02 mg/ml with a concentration of 0, 01 mg/ml, there is no significant difference.

Discussion

Collagen is a biopolymer that is well used in the field of tissue engineering and it have a high level of biodegradability.[9] Since, its high biodegradability, scaffold does not only support, but will unite with cells has a biological function as a medium for attachment, migration and proliferation of cells that can increase viability without affecting gene expression.[10]

The viability test aims to see the ability of cells to be able to live by showing cell responses in the short term and showing the ability of cells to survive from the process of apoptosis and necrosis triggered by the exposure of a material.[11] The viability test in this study uses a culture of bone marrow mesenchymal stem cell which is part of an adult stem cell that is able to make copies of cells that are identical to themselves for a long time and proliferate and differentiate into mature cells with morphological characteristics and certain functions for bone formation.[13]

Based on the results of this study, it appears in table 1 that the average absorbance value is higher than the average absorbance value of the cell control group. Bahi[14] states that the absorbance value of the treatment group which is smaller than the absorbance value of the control group indicates that the cell's ability to proliferate is low. If the absorbance produced is higher than the control, then the cell's ability to proliferate is high, but if the level of proliferation is too high, so it will result in cell death due to the possibility of changes in cell morphology.

In this study, the concentration of 0.01 mg/ml had the highest percentage of living cells of 116.502% and at a concentration of 0.32 mg/ml the lowest percentage of living cells was 93.184%. The research data is in accordance with the theory of loading-dose on drugs, i.e. the higher the dose given, the therapeutic effect on target cells is no better than the lower dose.[15] The absorbance obtained is influenced by several factors, including the type of solvent, pH, temperature, high electrolyte concentration, and the presence of intruders from external properties of fish that can survive at various water temperatures and pressures, making collagen extract of fish scales resistant to physical and chemical damage.[16]

Collagen derived from gouramy scales consists of various amino acids, especially the amino acid glycine. Collagen bonds from collagen extract of carp scales induce procollagen formation and cell proliferation[17]. The bonds that form type 1 collagen from gouramy scales consist of proline, lysine, glycine, and other amino acid components. Proline and lysine from type 1 collagen extract gouramy scales will form new collagen fibers.[18]

Collagen extract is rich in amino acids glycine, proline, glutamic acid, and aspartic acid and various kinds of peptides[17]. These components have the ability to actively regulate cell function. Glycine can regulate the proliferation and differentiation of progenitor cells, proline can induce differentiation of embryonic stem cells[19]. Glycine, glutamic acid, and alanine can influence signal transduction in the differentiation of osteoblastic bone marrow stem cells[20].

Type 1 collagen taken from gouramy scales can improve cell viability as indicated by the MTT test. The results of this study are in accordance with the research conducted by Zhang et al (2011) which states that Nano-fibrous type 1 collagen can support the growth of mesenchymal stem cells and is used for tissue engineering, and collagen material obtained from fish scales is used as engineering tissue as a potential candidate for bone collagen-based graft[22]. The conclusions of the results of this study are gouramy scales collagen soaked in the medium condition has good viability that is above 90%. It has the highest viability with an optimum dose of 0.01 mg/ml.

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