VIABILITY TEST OF FISH SCALE COLLAGEN (OSHPRONEMUS GOURAMY) ON BONE MARROW MESENCHYMAL STEM CELL CULTURE

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ABSTRACT

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 either from fish scales. This is what underlies the author to support engineering tissue used for the treatment of periodontal disease in the regenerative field by utilizing collagen derived from gouramy scales. As an initial step, the researchers wanted to conduct a study using collagen extract derived from gouramy scales (Osphoronemus gouramy) which was applied to bone marrow mesenchymal stem cell cultures to see viability in vitro. To determine the viability of collagen in carp (Osphronemus goramy) scales to bone marrow mesenchymal stem cells. 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 and hydrolyzed into a collagen hydrolysis solution 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. Collagen in carp scales can increase the viability of bone marrow mesenchymal stem cells with a percentage above 50% and the highest viability concentration at 0,01 mg / ml. The collagen of gouramy scales soaked in a medium condition has better viability than the collagen hydrolysis solution of carp. Collagen in carp 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

Periodontal disease is a disease that arises due to the body's response to the accumulation of bacteria in the periodontal tissue which causes the tooth supporting tissue to lose its collagen structure, and if this periodontal disease is not treated properly, it can cause tooth loss (Lumentut et al., 2013) .

The use of regenerative materials at the cellular level can stimulate the regeneration of periodontal tissue which requires organized cell proliferation, cell differentiation and the development of various cell types to form

periodontal attachments (Cahaya & Masulili, 2015).

The success of network engineering is largely determined by 3 factors that have been put forward by many previous researchers. The three factors are tissue engineering, namely scaffold, cells, and growth factors (Tabata, 2003). Scaffold serves to provide structure and substrate for tissue growth and development and will be degraded after healthy tissue grows (Meijer et al., 2007). Growth factors function as stimuli to carry out cell growth and differentiation within the scaffold (Murphy et al., 2013).

The role of type I collagen is as an extracellular protein matrix with the characteristics of increasing cell proliferation so that it directly affects the physiology and morphology of cells (Cardoso et al., 2014) Type 1 collagen can be obtained from fish scales.

Research on collagen derived from freshwater fish scales in Zhang et al's 2011 study showed that collagen derived from freshwater fish scales was safer to use than collagen sourced from other materials such as beef and pork, as well as collagen derived from aquatic fish scales. unsalted contain collagen type 1 which is quite high

METHODS

The type of research used is experimental laboratory. The design of this study used a post test only control group design. The technique of making gouramy scale collagen extract, gouramy scale collagen peptide solution, bone marrow mesenchymal stem cell cell culture technique, treatment technique, sterilization technique of gouramy scale collagen extract, use and sterility of laboratory equipment, research room temperature.

Statistical testing was carried out using Kolmogorov-Smirnov to determine the normality test. Homogeneity was calculated using Levene's Test. Testing The analysis was continued by using the One Way ANOVA statistical test at a significance level of 5% if the analysis was said to be homogeneous and the distribution was normal. If the analysis is not normally distributed and not homogeneous, then it is continued by using the Kruskal Wallis statistical test and followed by Mann-Whitney

RESULTS

The viability test was carried out using the MTT Assay method on bone marrow mesenchymal stem cell cultures with the concentration of peptide solution and gouramy scale collagen extract dissolved in medium conditions of 0.01 mg/ml, 0.02 mg/ml, 0.04 mg/ml. , 0.16 mg/ml, , 0.32 mg/ml . At 24 hours, it was observed that a purple color change in each well was observed and then the absorbance reading was carried out with the Elisa Reader, the following results were obtained:

Table 1 Optical Density in the treatment group of gouramy scale peptide solution and control

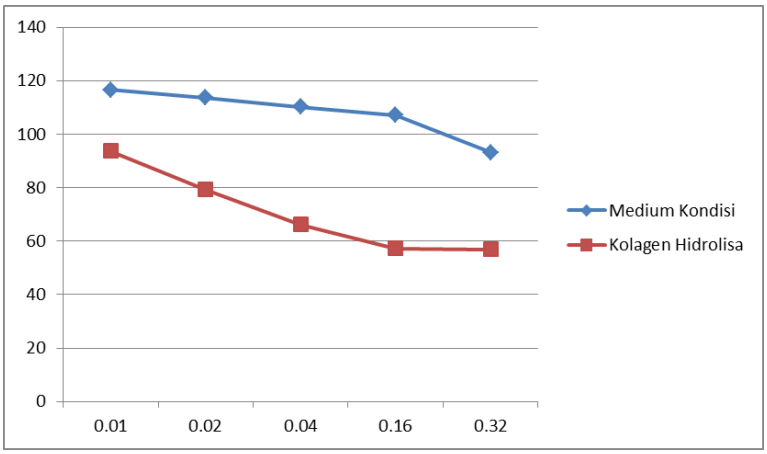
Kelompok	Rerata OD ± SD	Viabilitas Sel ± SD (%)
Kontrol Sel	0,787 ± 0,033451	100 ± 0,006422
0,01 mg/ml	0,743 ± 0,041613	93,686 ± 0,02638
0,02 mg/ml	0,642 ± 0,059601	79,301 ± 0,09447
0,04 mg/ml	0,551 ± 0,042876	66,136 ± 0,03663
0,16 mg/ml	0,488 ± 0,065944	57,176 ± 0,05548
0,32 mg/ml	0,487 ± 0,012662	56,952 ± 0,02836

Table 2 Optical Density in the treatment group of gouramy scale collagen extract dissolved in medium conditions

Kelompok	Rerata OD ± SD	Viabilitas Sel ± SD (%)
Kontrol Sel	0,787 ± 0,033451	100 ± 0,00642
0,01 mg/ml	0,902 ± 0,008386	116,502 ± 0,06263
0,02 mg/ml	0,882 ± 0,051046	113.632 ± 0,07160
0,04 mg/ml	0,858 ± 0,043432	110.188 ± 0,06837
0,16 mg/ml	0,837 ± 0,151168	107.175 ± 0,24156
0,32 mg/ml	0,739 ± 0,047455	93,184 ± 0,05863

From the table the results of the calculation of the percentage of the number of living cells can be made a graph as follows:

Figure 1 Percentage of live cells from the group treated with peptide solution and gouramy scale collagen extract



According to the graphs and tables presented, it was found that the percentage of live cells of gourami scale collagen dissolved in the medium had a higher percentage of live cells than the collagen peptide solution. In each preparation, the highest increase in the number of cells was found at a concentration of 0.01 mg/ml with a percentage of live cells as much as 116.502% in the gouramy scale collagen extract dissolved in the medium and 93.686% in the collagen peptide solution. The percentage of live cells reflects the value of cell viability in the sample. The increase in the number of bone marrow mesenchymal stem cells was proven by the comparison of the average percentage of survival for the control sample group, which was 100%. Then the value of control viability and the highest treatment viability will be tested for normality, homogeneity test, and different test to see the significance.

DISCUSSION

The bone regeneration process requires osteoprogenitor cells combined with osteoinductive biomaterials to trigger the differentiation of other cells to form new bone. Various studies have shown that suitable biomaterials will be able to induce a certain microenvironment for cells to exhibit the desired behavior (Yang et al., 2011).

Collagen as a scaffold not only supports, but will unite with cells because it is biodegradable. Collagen has a biological function as a medium for attachment, migration and proliferation of cells that can increase viability without affecting gene expression (Khan, 2009).

The production of collagen using the basic ingredients of gouramy fish scales by combining two methods of collagen extraction, namely ASC (Acid Solubility Collagen) and PSC (Pepsin Solubility Collagen) and carried out the lyophilization process using a freezedrying tool to remove the water content in the extract of gouramy scales.

The viability test was determined by 3-(4,5-Dimthylthianizol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT). This assay is based on the reduction (reduction) of the yellow tetrazolium salt to purple formazan crystals by the mitochondrial enzyme succinate dehydrogenase which is secreted from the mitochondria of metabolically active cells.

The viability test in this study used bone marrow mesenchymal stem cell cultures, which are part of adult stem cells that are capable of proliferating and differentiating into mature cells with certain morphological characteristics and functions for bone formation (Herzog et al., 2003).

Based on the results of the study in table 1, the treatment group of gouramy scale collagen peptide solution obtained the average absorbance value of the treatment group was smaller than the

average absorbance value of the control cell group. On the other hand, in table 2, the average absorbance value of the gouramy scale collagen extract treatment group immersed in medium conditions was higher than the average absorbance value of the control cell group. Thus, the viability of bone marrow mesenchymal stem cells in the treatment group of gouramy scale collagen extract immersed in medium conditions was greater than that of the gouramy scale collagen peptide solution treatment group.

In table 1, it can be seen that the percentage of living cells or the highest viability value of the treatment group of gouramy scale collagen peptide solution is at a concentration of 0.01 mg/ml as much as 93.686% and has the lowest percentage of living cells at a concentration of 0.32 mg/ml as much as 56.952 %.

In table 2, the treatment group of gouramy scale collagen extract soaked in medium conditions had the highest percentage of live cells at a concentration of 0.01 mg/ml at 116.502% and had the lowest percentage of living cells at a concentration of 0.32 mg/ml at 93.184%. So, the highest viability value of peptide solution and gouramy scale collagen extract soaked in medium conditions at a concentration of 0.01 mg/ml and the lowest at a concentration of 0.32 mg/ml and from these data it can also be concluded that the viability value of the collagen extract solution gouramy scales soaked in the medium was higher than the collagen peptide solution of gouramy scales.

Based on the average percentage of living cells, all treatment groups of fish scale collagen peptide solution and gourami scale collagen extract soaked in medium conditions had live cell percentage > 50%. According to the Telli standard, a substance is said to be non-toxic if the percentage of living cells after exposure to the substance is more than 50% (Meizarini, 2005). From the results of the percentage of living cells, it can be seen that the optimum dose of the treatment group of fish scale collagen peptide solution and gouramy scale collagen extract soaked in medium conditions was 0.01 mg/ml.

In this study, -MEM medium was added with 10% FBS, Penicillin, 2% Streptomycin, 0.5% fungizone to soak the collagen extract and bone marrow mesenchymal stem cell cultures. The success of the expansion can be seen from the level of proliferation and the characteristics of the bone marrow mesenchymal stem cells produced. The choice of culture medium and supplements added to the culture medium such as growth factors can affect cell proliferation and maintain the pluripotency of stem cells (Hagmann et al., 2013).

The significance test using the Kruskal-Wallis test found significant differences in all treatment groups of gouramy scale collagen peptide solution and gouramy scale collagen extract soaked in medium conditions. In the treatment group of gouramy scale collagen peptide solution, control cells were found to be in the highest rank because the absorbance value of control cells was higher than the treated group.

External collagen derived from gouramy fish scales consists of various amino acids and is dominated by the amino acid glycine. Besides

glycine, there are amino acids proline and lysine which are other essential components for the formation of collagen, namely type 1 collagen because they are involved in telopeptide cross-linking bonds in collagen.

Collagen extract is rich in the amino acids glycine, proline, glutamic acid, and aspartic acid as well as various peptides. (Liu & Jiao, 2014) have reported that small collagen fragments can bind to the 2A integrin domain which is a receptor of collagen. The 2A domain of the integrin (α2A) exhibits specific binding to the triple-helical collagen fragment.

Type 1 collagen taken from gouramy fish scales can affect cell viability as indicated by the MTT test. Type 1 nanofibrous collagen can support the growth of mesenchymal stem cells and is used for tissue engineering. Collagen derived from gourami scales is non-toxic and can be used as a potential candidate for collagen-based bone graft.

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