VIABILITY TEST OF FISH SCALES COLLAGEN GOURAMI (OSPHRONEMUS GORAMY) ON HUMAN GINGIVAL FIBROBLASTS CELLS

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ABSTRACT

Periodontal disease is a pathological inflammatory condition of the periodontal tissues surrounding the teeth, including Human Gingival Fibroblasts (HGF) which is one of the major components of tissue formation in periodonsium. HGF regeneration with the accelerating proliferation of tissue engineering therapy needs. Generally, the tissue engineering using regenerative materials from cow or pig as the therapies, but these materials have some flaws so this research to find alternative materials regenerative tissue engineering scaffold collagen type 1 derived from fresh water fish scales, one of which are gourami fish scales. This research was conducted to test the viabilityof fish scales collagen gouramy against Human Gingival Fibroblasts for 24 hours. This study aimed to determine the concentration of fish scale collagen gourami which can maintain the viability of human gingival fibroblast cells for 24 hours. HGF is taken from healthy gingiva and planted in 96 well plates. Fish scales collagen gouramy with a concentration of 0.32 mg/ml, 0.16 mg/ml, 0.04 mg/ml, 0.02 mg/ml and 0.01 mg/ml were added to each well and incubated during 24 hours. MTT Assay is performed to see the viability of fibroblast cells. The viability of HGF were increased after the addition of fish scales collagen gourami on concentration 0.32 mg/ml until 0.01 mg/ml. The highest viability of the cells was shown after the addition of 0.01 mg/ml. Fish scales collagen gouramy has the potential in tissue engeneering and the concentration of 0.01 mg/ml shows the highest viability of HGF.

Keyword: Fish Scales Collagen Gourami, Scaffold, Collagen, HGF.

INTRODUCTION

Periodontal disease is a lesion of the oral cavity that causes the tooth supporting area to lose its collagen structure which is a response to the accumulation of bacteria in the periodontal tissue (Lumentut, 2013). Periodontal disease is one of

the dental and oral diseases with a high prevalence rate and is a disease in the oral cavity that affects almost all humans in the world and reaches 50% of the adult population (Newman et al., 2012). In Indonesia, the prevalence of periodontal disease in all age groups reaches 96.58% (Tampubolon, 2010).

Tissue engineering is seen as regenerative dentistry, this is because the goal of tissue engineering is to restore tissue function through the delivery of stem cells, bioactive molecules, or synthetic tissue constructs engineered in the laboratory (Kumar et al., 2011). The tissue engineering approach to bone and periodontal regeneration combines three key elements to promote regeneration, namely progenitor cells, scaffold or support matrix and signaling molecules (growth factors).

Scafold serves to provide structure and substrate for tissue growth and development and will be degraded after healthy tissue grows (Gert et al., 2007). Collagen is a connective tissue protein with the basic molecule forming collagen, namely three polypeptide chain units that are twisted together to form a triple helical structure which is better known as tropocollagen (Gelse et al. 2003).

Fish is one of the biota that can be used as raw material for producing collagen. Collagen derived from fish skin and bones has a smaller molecular structure compared to collagen made from beef or pork so it is easier to absorb (Kumar et al., 2011). Based on research by (Nagai et al., 2004), the components contained in fish scales include 70% water, 27% protein, 1% fat, and 2% ash. Organic compounds consist of 40-90% in fish scales and the rest is collagen.

This research is more focused on freshwater fish, namely gouramy, scales and bones derived from gouramy fish which have more numbers on the surface of the fish body than other freshwater fish. This research is to develop and determine the potential of gouramy fish scales as an alternative to scaffold in tissue engineering. As an initial stage, this study aimed to examine the viability of gouramy (Osphronemus goramy) scale collagen against Human Gingival Fibroblast (HGF) cells.

METHODS

This research is an experimental laboratory with a post-test only control group design. One of the methods used in the viability test. The principle of this method is a redox reaction that occurs in the cell. MTT [3-(4,5-dimethylthiazol-2yl)- 2,5-diphenyl tetrazolium bromide] was reduced by the enzyme succinate dehydrogenase present in the mitochondria of living cells to produce a purplish blue formazan product which was insoluble in water. The formazan produced can be calculated by dissolving DMSO (Dimethyl sulfoxide) and measuring the optical density of the resulting solution. The blue-purple color reaction was used as a measure of the number of living cells with the help of an ELISA reader at a wavelength of 590 nm.

RESULTS

The absorbance results of Human Gingival Fibroblast (HGF) cells after administration of gouramy scale collagen preparations of peptide and extract solutions obtained the following results:

Table 1. Absorbance results of the treatment group of gouramy scale collagen peptide solution against Human Gingival Fibroblast (HGF) cells.

REPLIKASI	KONSENTRASI					
	Kontrol Sel	0,32 mg/ml	0,16 mg/ml	0,04 mg/ml	0,02 mg/ml	0,01 mg/ml
1	0,552	0,840	1,010	0,825	0,928	0,816
2	0,678	0,857	0,793	0,890	0,842	0,908
3	0,659	0,822	0,950	0,941	0,902	0,940
4	0,648	0,810	0,638	0,856	0,854	0,965
Absorbansi rata-rata	0,634	0,832	0,848	0,878	0,882	0,907

In table 1, it can be seen that the average absorbance value of the control cell group is 0.634. The average absorbance of the gouramy scale collagen peptide solution in the 0.32 mg/ml concentration group was 0.832, the gouramy scale collagen peptide solution in the 0.16 mg/ml concentration group was 0.848, the gouramy scale collagen peptide solution in the 0.04 mg concentration group /ml was 0.878, the concentration of gouramy fish scale collagen peptide solution was 0.02 mg/ml group was 0.882 and the concentration group of gourami fish scale collagen peptide solution was 0.01 mg/ml was 0.907.

REPLIKASI	KONSENTRASI					
	Kontrol Sel	0,32 mg/ml	0,16 mg/ml	0,04 mg/ml	0,02 mg/ml	0,01 mg/ml
1	0,552	0,725	0,779	0,750	0,639	0,923
2	0,678	0,774	0,704	0,804	0,827	0,898
3	0,659	0,660	0,795	0,766	0,762	0,745
4	0,648	0,726	0,760	0,745	0,855	0,795
Absorbansi rata-rata	0,634	0,721	0,760	0,766	0,771	0,840

Table 2. The absorbance results of the gouramy scale collagen extract treatment group against Human Gingival Fibroblast (HGF) cells.

In table 2, the average absorbance value of the control cell group is 0.634. The average absorbance of gouramy scale collagen extract in the 0.32 mg/ml concentration group was 0.721, the gouramy scale collagen extract in the 0.16 mg/ml concentration group was 0.760, the gouramy scale collagen extract in the 0.04 mg/ml concentration group was 0.766, group gourami fish scale collagen extract. the concentration of 0.02 mg/ml was 0.771 and the collagen extract of gouramy fish scales in the 0.01 mg/ml concentration group was 0.840.

After obtaining the absorbance results from each well. Furthermore, the percentage of the number of living cells in each well and the average of each concentration were calculated, the calculations were carried out using the following formula (Reddy et al, 2011):

$$Presentase\ sel\ hidup = \frac{(\text{absorbansi perlakuan} - \text{absorbansi kontrol media})}{(\text{absorbansi kontrol sel} - \text{absorbansi kontrol media})} X100\%$$

Table 3 Viability value of the treatment group of gouramy scale collagen hydrolysis solution against Human Gingival Fibroblast (HGF) cells

Konsentrasi	Presentase sel hidup rata-rata (%)
Kontrol Sel	100
0,32 mg/ml	136,347
0,16 mg/ml	139,192
0,04 mg/ml	144,745
0,02 mg/ml	145,388
0,01 mg/ml	150,115

In table 3, it can be seen that the percentage of living cells in the group of gouramy scale collagen peptide solution treatment had an average percentage of 136.347%; 139.192%; 144.745%; 145.388%; 150.115%. The concentration of 0.01 mg/ml had the highest average percentage of living cells, at 150.115%, while the concentration of 0.32 mg/ml had the lowest average percentage of living cells, which was 136.347%.

DISCUSSION

Periodontal tissue has many important building blocks such as collagen and fibroblasts. Fibroblast cells function as producers of connective tissue. The fibers produced by fibroblast cells include collagen fibers, reticulum fibers, oxytalan fibers, and elastic fibers. Fibroblast cells secrete cytokines and several growth factors, including stimulating cell proliferation and inhibiting the differentiation

process (Juwita, Harlystiarini et al., 2010).

Gouramy scale collagen was formed into two preparations, namely peptide solution and gouramy scale collagen extract. The gouramy scale collagen extract was hydrolyzed to produce a solution of gouramy scale collagen peptides. Hydrolyzed collagen is obtained by chemical or enzymatic hydrolysis under controlled conditions (Schrieber & Gareis, 2007; Wang et al., 2009).

Based on the research results of the treatment group with the gouramy scale collagen peptide solution, it was found that the average absorbance value of the treatment group was greater than the average absorbance value of the control cell group. Furthermore, the average absorbance value of the gouramy scale collagen extract treatment group was greater than the average absorbance value of the control cell group. So, the average value of absorbance of Human Gingival Fibroblast (HGF) cells in the treatment group of collagen peptide solution and gourami scale collagen extract was greater than the treatment group of each control cell.

The percentage of live cells or the highest viability value from the treatment group of gouramy scale collagen peptide solution was at a concentration of 0.01 mg/ml as much as 150.115% and had the lowest percentage of living cells at a concentration of 0.32 mg/ml as much as 136.347%. Meanwhile, in the treatment group, gouramy scale collagen extract had the highest percentage of live cells at a concentration of 0.01 mg/ml at 137.816% and the lowest percentage of living cells at a concentration of 0.32 mg/ml was 115.971%. So, the highest viability value of the peptide solution and gouramy scale collagen extract was at a concentration of 0.01 mg/ml and the lowest was at a concentration of 0.32 mg/ml and from these data it can also be concluded that the viability value of the gouramy scale collagen peptide solution was higher. than the viability value of the gouramy scale collagen extract.

The results showed that there was a gradual increase in the viability value of the peptide solution and gouramy scale collagen extract from the highest concentration (0.32 mg/ml) to the lowest concentration (0.01 mg/ml). The research data showed that there was a significant difference from all treatment groups of gouramy scale collagen peptide solution against the control cells. While in the treatment group, the gouramy scale collagen extract only had a significant difference at the lowest 3 concentrations, namely the concentration of 0.04 mg/ml, 0.02 mg/ml 0.01 mg/ml, and had no significant difference at a concentration of 0, 16 mg/ml and 0.32 mg/ml.

Viability after administration of gouramy scale collagen extract with concentrations of 0.16 mg/ml and 0.32 mg/ml to approach the viability value of control cells. Exposure to gouramy scale collagen extract with a concentration of 0.16 mg/ml had an average live percentage of 122.992% and a concentration of 0.32 mg/ml had a live cell percentage of 115.971%. The viability value of the 0.32 mg/ml concentration that was read was only 15.97% compared to the control cells, which was 100%. This indicates that there was a plateau effect on exposure to

concentrations of 0.16 mg/ml and 0.32 mg/ml, so that exposure to these concentrations did not significantly affect cell proliferation. The research data are in accordance with the loading-dose theory of drugs, namely the higher the dose administered, the therapeutic effect on target cells is not better than at lower doses (McCormack et al., 2011).

The composition of the scaffold consists of poly(alpha-hydroxyl) polymers which can be degraded in the body (PLA, PGA, PLGA) and natural polymers. Natural polymers are divided into proteins (collagen, silk, fibrinogen, elastic, creatine, or actin), polysaccharides (cellulose, amylose, dextran, chitin (chitosan), and glycosaminoglycans), and polynucleotides (DNA and RNA) (Ratner, 2004).

Collagen derived from carp scales can be considered as an alternative material for tissue engineering because of its abundant collagen content and good biocompatibility (Kittiphattanabawono et al., 2005). This study is also in accordance with the statement of Brett D in 2008 that collagen is able to accelerate the wound healing process by forming an extracellular matrix for the attachment of cells forming periodontal tissue, especially fibroblast cells.

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