Addition of Gourami Fish Scales to Glass Ionomer Cement (GIC) Increases Odontoblast like Cells Number and Expression of IL-10 (Interleukin-10)

[1]Roedy Budirahardjo*, [2]Dyah Setyorini, [3]Sulistiyani, [4]I Dewa Ayu Ratna Dewanti, [5]Eri Hendra Jubhari

[1.2.3]Department of Pediatric, Faculty of Dentistry, Jember University, Jember, Indonesia
[4]Department of Biomedic, Faculty of dentistry, Jember University, Jember, Indonesia
[5]Department of Prosthodontic, Faculty of Dentistry, Hasanudin University, Makasar, Indonesia

Correspondence: Roedy Budirahardjo, Faculty of Dentistry, Jember University. Kalimantan street 37 Jember. No HP.

ABSTRACT

Introduction. Fish scales are usually not used anymore or thrown away, in fact they contain organic and inorganic materials that are also found in bone and dentin. **Purposed**. This research to analyze the addition of gouramy scales powder (GSP) to Glass Ionomer Cement (GIC) on odontoblast count and IL-10 expression in the oral cavity of Wistar rats. **Method**. we used male Wistar rats C: group mice that did not bore holes in their teeth. Groups in this research, P1: the sample is filled with GIC. P2: GIC + 2.5% GSP. P3: GIC + 5% GSP. P4: GIC + 10% GSP. After 7 days the rats were sacrificed and their teeth were made preparations for odontoblast cells observation (light microscope magnification 400 times) and analysis of IL-10 by immunohistochemical technique. **Results.** The ANOVA and LSD test confirmed significant differences. The increase in the number of odontoblasts and IL-10 is thought to be due to goramy scales caused by chitin, calcium, amino acids, omega 3, and omega 6. **Conclusion**. The addition of gouramy scales powder (GSP) to Glass Ionomer Cement (GIC) increased the number of odontoblasts like cells and the expression of IL-10 in the oral cavity of Wistar rats. So that the addition of gouramy fish scales can be considered to improve the quality of GIC.

Keywords: Glass Ionomer Cement; gouramy fish scales; Odontoblasts like cells; Interleukin; Immunohistochemistry

I. INTRODUCTION

Nowadays, various efforts are made to find dental filling materials that come from nature, but have a quality that is not inferior to materials on the market. One of the materials that have a similar composition to teeth and alveolar bone is fish waste (fish scales). In the future, it is hoped that new materials will be created that have better performance, are stronger, faster and more efficient. Before a patented material is created, it must be biocompatible and non-immunogenic. Our previous research proved that powder and paste of thorns and fish scales (Gurami and Kuniran) can inhibit the growth of bacteria that cause dental caries (Streptococcus mutans), In addition, we also proved that fish scales are non-immunogenic (in vitro) ((Dewanti et al, 2019). Therefore, we assume that the presence of organic and inorganic materials in fish scales that are similar to bones and teeth can be used as fillings/tooth fillings. To be used as a dental filling material, it must have good physical and chemical properties. from in vivo trials is the first step between in vitro tests and clinical trials in humans.

Fish waste (fish spines and scales has the same composition as dentin and bone and is known to contain a lot of protein (32%), in addition to its collagen content. Fish spines and scales are also a source of calcium (Nie, 2014; Rotland et al., 2005). Fish scales consist of type-I Fibril collagen, and are partially mineralized with hydroxyapatite (16-59% mineral content). basal " or 'collagen' layer) (Dzu et al., 2011). Gouramy scales consist of collagen type 1 fibrils and mineralization with hydroxyapatite (16% to 59% mineral content).The outer layer (bone layer) of fish scales is more mineralized, containing higher inorganic materials, while the inner layer (basal/collagen layer) is higher organic materials content (Dzu et al., 2011; Dewanti et al., 2020). 3, omega 6, an d flavonoids which have antibacterial properties (Dewanti et al, 2019). Gouramy Fish (Osphronemus gouramy) powder Scales reduce the marginal gap, increase compressive strength and inhibit the growth of Streptoccus mutans and Candida albicans (Dewanti et al. 2022).

We hope that fillings derived from fish scales minimize the side effects of the filling materials used, in addition to being biocompatible, anti-bacterial, preventing secondary caries, and stimulating the formation of dental tissue. The formation of dental tissue, among others, is characterized by the formation of odontoblast cells. In addition, fish scales also have an anti-inflammatory effect which is characterized by an increase in IL-10.

This research to analyze the addition of gouramy scales powder (GSP) to Glass Ionomer Cement (GIC) on odontoblast cells count and IL-10 expression in the oral cavity of Wistar rats.

II. METHOD

2.1. Making Gourami Fish Scales

Making fish scales powder using the freeze drying method, where this process begins by freezing the fish scales at a temperature of -40°C. Drying is through a sublimation mechanism, where the frozen fish scale powder is put into a freeze drying device with a pressure of 0.036 psi and the temperature is raised in a controlled manner to 38°C. Drying ends when the water content in the fish

scales has sublimated. Blended using a blender. Next, the fish scales powder was sieved using a sieve with a size of 200 mesh (Hariyadi 2017; Dewanti et al, 2022).

2.2. Mixing fish scale powder with GIC

Manipulate the mixture of GIC powder and fish scale powder according to the calculated ratio with GIC liquid on a paper pad by mixing half of the powder with liquid and stirring using an agate spatula for 15-20 seconds with a rolling motion. Next, mix in the remaining half of the powder and stir for 15-20 seconds in a rolling or folding motion. The result of manipulation must have a puttylike or putty-like consistency and a glossy surface

2.3. Test animal

20 male Wistar rats, aged 12-14 weeks, weight 250 which had been adapted for 7 days, were divided into 5 groups. C: sample that did not make cavities in its teeth, P1: the sample is filled with GIC, P2: GIC + 2.5% GSP, P3: GIC + 5% GSP, P4: GIC + 10% GSP. After 7 days the rats were sacrificed and preparations were made from their teeth.

2.3. Odontoblast observation

The tissue was fixed using 10% formalin solution for at least 12-18 hours, then decalcified. Decalcification was carried out using 10% formic acid solution for 7 days. Dehydration using alcohol in low to high concentrations. Clearing is a tissue cleaning process using clearing materials. Materials that can be used include: xylol, toluene, and benzene. The tissue is wrapped in filter paper and then put into the embedding material, namely paraffin TD 56-60°C. Embedding using paraffin. Paraffin block incision using a microtome. Painting using Hematoxylin-Eosin (HE). Subsequently, the odontoblast cells were observed using a 400 times magnifying light microscope.

2.4. Analysis of IL-10 by immunohistochemical technique.

The preparations were deparaffinized, washed (2x PBS) and blocked with 3% BSA for 10 minutes. Next, it was reacted with anti IL-10 mouse antibody (Dako), incubated 24 hours at 40C, then reacted with secondary antibody (Dako). Washed 3 times with PBS (Phosphate buffered saline), added peroxidase labeled streptavidin and incubated for 1 hour. The preparation was added with Trek Avidin-HRP (horseradish peroxidase) reagent, washed with PBS, preparation with DAB chromogen substrate (Diamonobenzinidine / Daco). Washed with distilled water, added Meyer-HE, washed, given entelan and covered with a lid. Data were obtained by counting leukocytes expressing cytokines (brown color) under a light microscope with 400x magnification per 5 visual fields.

III. RESULTS

The normality test uses the Shapiro Wilk test, where if p> 0.05 then the data is normally distributed. homogeneity test using Levene's test, where if p> 0.05 then it shows the data group comes from a population that has the same variance (homogeneous). The ANOVA and LSD test confirmed significant differences.

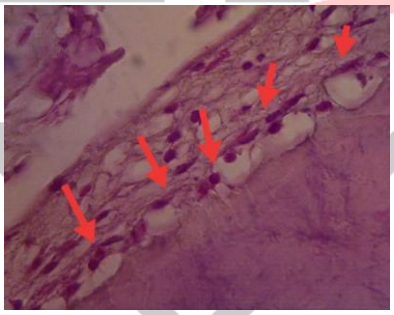

Figure 1. Odontoblast like cells (red arrow). Observation using a light microscope magnification 400 times

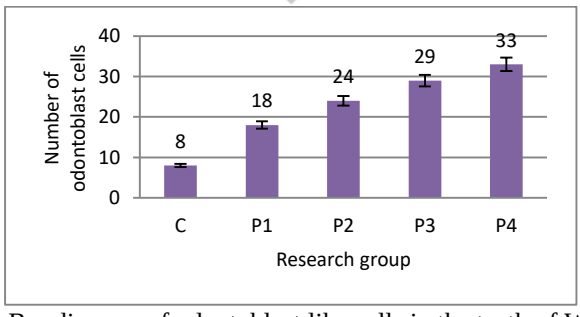

Figure 2. Bar diagram of odontoblast like cells in the teeth of Wistar rats

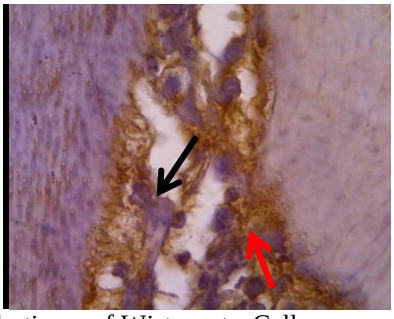

Figure 3. Expression of IL-10 in the dental pulp tissue of Wistar rats. Cells expressing IL-10 (red arrow), cells not expressing IL-10 (black arrow). Observations using a light microscope with a magnification of 400 times.

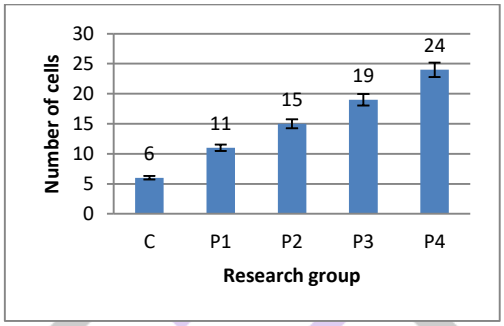

Figure 4. Bar diagram of Il-10 expression in the pulp tissue of Wistar rats

IV. DISCUSSION

The addition of gouramy fish scales to GIC is able to act as an anti-inflammatory and can increase the number of odontoblast cells. The increase in the number of odontoblasts and IL-10 is thought to be due to goramy scales caused by chitin, calcium, phosphorus, amino acids, omega 3, and omega 6.

Omega 3, omega 6, immunomodulating amino acids in this case are anti-inflammatory. Omega-3 (ω-3) and omega-6 (ω-6) are polyunsaturated fatty acids (PUFA) that have effects on various chronic inflammatory and autoimmune diseases through various mechanisms, including modification of cell membranes. lipid composition, gene expression, cellular metabolism, and signal transduction (Balic et al., 2020; DiNicolantonio et al., 2020).

Phosphorus in all cells and tissues is an important component of DNA, RNA and phospholipids, a source of high-energy binding in adenosine triphosphate (ATP), a substrate for various kinases and phosphatases, and a regulator of intracellular signaling. The role of Phosphorus is reinforced by calcium (Ca2+), the most abundant mineral, in hydroxyapatite (HAP) crystals deposited in the matrix. Other mineral tissues such as teeth also contain calcium phosphate as HAP. Therefore, the maintenance of "normal" phosphate homeostasis (inorganic or orthophosphate, Pi) is essential for the development, maintenance, and repair of teeth (Yıldırım, 2007; Foster, 2008).

Methionine is an essential sulfur-containing amino acid that is not nucleophilic and will react with several electrophilic centers. Its function is to eliminate toxins, improve cardiovascular health, assist the liver in processing fats, make creatine (a natural nutrient in muscles, heart and blood vessel function), formation of nails, skin and connective tissue, reduce inflammation. Proline is a non-essential amino acid for the production of collagen and cartilage, keeping muscles and joints flexible, cell production. Glutamine is one of three amino acids in the important antioxidant compound glutathione, used as a metabolic raw material by leukocytes and erythrocytes. essential nutrients for lymphocyte proliferation, cytokine production, killing activity by macrophages and neutrophils, fibroblasts, apoptotic neutrophil protection. Glycine acts as an anti-inflammatory (Herwald et al., 2016; Afonina et al., 2017; Cruzat et al., 2018; Ma et al., 2018; Dewanti et al., 2019; Ginwala et al., 2019).

The formulation of gouramy fish scales which is rich in chitin, calcium, phosphate will stimulate dentinogenicity by a mechanism by gourami fish scales which are thought to easily absorb leaving apatite rich and replaced with reparative dentin. The absorbed HA will be more and more slowly create nucleation centers for mineralization of new dentin tissue in the inflamed pulp tissue. In addition, the healing of inflamed pulp tissue is also supported by the presence of amino acids that act as anti-inflammatory. Therefore, GIC added with gourami fish scales showed a decrease in IL-10 which acts as an anti-inflammatory cytokine.

v. CONCLUSION

The addition of gouramy scales powder (GSP) to Glass Ionomer Cement (GIC) increased the number of odontoblasts like cells and the expression of IL-10 in the oral cavity of Wistar rats. So that the addition of gouramy fish scales can be considered to improve the quality of GIC.

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