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EFFECTS OF USING HYDROXIAPATITE-GELATIN GEL AND ROSELLA EXTRACT TO PREVENT RELAPSE IN ORTHODONTIC TREATMENT

Sabrina Fitri Auliadini
University of Prof. Dr. Moestopo (Beragama), Jakarta, Indonesia
Evie Lamtiur Pakpahan*
University of Prof. Dr. Moestopo (Beragama), Jakarta, Indonesia

*Correspondence: evie.l@dsn.moestopo.ac.id

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ABSTRACT

To correct malocclusion and malposition in dentistry using orthodontic treatment. Teeth that have moved through the bone with orthodontic devices tend to return to their original position or what is usually called relapse. Relapse is when the teeth return to their original position before orthodontic treatment was carried out. Relapse prevention can be achieved using mechanical devices but there are still some limitations. The use of scaffolds can be a solution in preventing tooth relapse because scaffolds can support cells to grow and develop into new bone in the damaged area. The scaffold components are hydroxyapatite, gelatin, and rosella flower extract which contains flavonoids to inhibit bone resorption. To find out whether these components are toxic or not before they are applied to the human body, a cytotoxicity test is carried out, which is a laboratory study. This research was carried out by incubating the hydroxyapatite scaffold, gelatin and rosella flower extract with osteoblast cells for 24 hours to see whether the rosella extract was toxic to osteoblast cells. Conclusion: This toxicity test shows that rosella flower extract as an ingredient in making scaffolds is not toxic to osteoblast cells.

INTRODUCTION

Public concern for aesthetics, especially regarding teeth, in the modern era is very high. The Global Burden of Disease Study says nearly 3.5 billion people in the world experience dental and oral health problems. Dental and oral health problems among the Indonesian population in 2013 - 2018 increased from 25.9% to 57.6%, which means that lifestyle and trends influence a person to obtain aesthetic value by undergoing dental treatment, namely orthodontic treatment (Suala, Wibowo, & Setyawardhana, 2021). Teeth that have been moved through the bone using orthodontic tools or appliances show a tendency to return to their original position because orthodontic treatment causes structural changes in the alveolar bone and periodontal ligament fibers. Tooth movement will produce two zones, namely the compression zone and the tension zone. The compression zone causes alveolar bone density to decrease due to increased resorption activity by osteoclasts, while in the tension zone there is a remodeling process carried out by osteoblasts and stretching of the periodontal ligament fibers. Post-

orthodontic treatment affects the alveolar bone and periodontal ligament fibers in an unstable condition. This happens because the tissue remodeling process takes more than 4 months. If this condition is ignored, it will cause the condition to recur. We know this event as relapse or instability (Nugraha et al., 2019).

Relapse is a condition that occurs after orthodontic treatment and is characterized by the return of part or all of the condition as before orthodontic treatment. Relaps is a response of the tooth supporting tissue due to the large force exerted on the tooth and can contribute to the stability of the occlusion and increase the mechanical stress through the transseptal fiber system (Rajagukguk, Sagita, Hermawan, & Pakpahan, 2023). The prevalence of relapse after orthodontic treatment is quite high, namely 61.5% (R. Sutjiati, Rubianto, I. B. Narmada, I. K. Sudiana, 2017). The high rate of relapse in patients after active orthodontic treatment was found in several studies, causing relapses of less than 1 mm to be found in 89% of cases and relapses of less than 3 mm to be found in 11% of cases. According to Proffit, although the patient may feel that the treatment has been completed when the appliance is removed, the teeth may still be in an unstable position resulting in continuous pressure from the surrounding soft tissue may result in a tendency to relapse.

There are several etiologies that cause relapse, namely relapse due to periodontal and gingival tissue factors, this tissue maintains the balance between teeth and soft tissue which is needed during the tissue remodeling period after orthodontic treatment. When teeth are moved orthodontically, the periodontal tissue and gingival tissue surrounding the teeth will stretch, and the stretched tissue will shorten, potentially causing tooth relapse. The next causes of relapse are growth factors, bone adaptation, muscle pressure, failure to eliminate causative factors, bad habit factors, and soft tissue pressure (Edrizal, Busman, & Azmir, 2021).

To stabilize the correction and hold the teeth in the position that has been achieved, a tool is used, namely a retainer. However, retainers have several disadvantages, namely that they can cause a slight open bite, continuous use can cause discoloration, cause an unpleasant odor, can break when exposed to occlusion pressure, and research conducted by Shanghai shows that the incidence of vacuum formed retainers breaking is 24%. One study showed that 50% of relapses were seen 2 years after retainer use, 28% of relapses were seen 2-5 years after retainer use, and 12% of relapses were seen 5-10 years after retainer use (Dianastesi & Utari, 2016; Goenharto & Rusdiana, 2015).

The use of bone graft scaffolds can be one solution to answer the problems faced in the bone repair process. A scaffold is a very important component because it can support cells to grow and develop into new bone tissue in the damaged area with the desired shape and size (NRP, 2021). The components used are hydroxyapatite which has been used in bone tissue engineering for many years as a good bone replacement and replacement regeneration material so it has great potential for bone regeneration, gelatin which has a strong bonding affinity with hydroxyapatite so it can resemble real bone and can provide porous structure to increase cell attachment and growth, as well as rosella flower extract which has the potential to treat bone damage (Razali et al., 2014).

Hydroxyapatite (HAp) is a porous scaffold that closely resembles bone consisting of approximately 50% human bone and 70% - 80% dentin and enamel. The surface chemistry of HA scaffolds is critical in the development of new bone. Minor substitutions with different ions have a significant influence on the structure and biocompatibility of HA because the ability of cells to migrate is critical for new bone growth influenced by external cellular proteins (Ra, Pg, & Gl, 2019). Gelatin is

denatured collagen which is often used in the pharmaceutical and medical fields. As a biomaterial, gelatin has high tensile strength and controllable biodegradation, low inflammatory and cytotoxic effects. Gelatin has been used in tissue engineering and as a bone graft base material. This material has been used in tissue engineering because it can produce three-dimensional scaffolds for bone tissue regeneration because its physicochemical properties can be manipulated well. Gelatin has been used as a composite matrix due to its very similar composition, and hydroxyapatite (HAp) is used as a good filler to strengthen composite materials. The HAp nanoparticle content in the gelatin/HAp composite will increase cell attachment, proliferation, and increase alkaline phosphatase levels (Nawafi, Masruroh, & Santjojo, 2022).

This research has never been carried out before, so it is hoped that this research can determine the effectiveness of using scaffolds with biomaterials such as hydroxyapatite, gelatin, and rosella flower extract (Hibiscus sabdariffa L.) in preventing recurrence after orthodontic treatment and can determine the percentage of living cells after osteoblast cells are injected by rosella flower extract.

The effect of adding rosella flower extract on osteoblast cell viability and the mechanical properties of hydroxyapatite, gelatin and rosella flower extract scaffolds needs to be known whether they meet the criteria required for bone graft. Bone graft is a surgical procedure to replace missing bone with material from the patient's own body, artificial, synthetic, or natural. Bone grafting is possible because bone tissue has the ability to regenerate completely if it is provided with space to grow. Bone graft must have three basic functions, including osteogenesis, osteoconduction and osteoinduction. Bone graft must be bicompatible, that is, it can be accepted by the body, have good mechanical properties, and be easy to manipulate. In supporting more effective bone cell formation, tissue engineering introduces a combination of cells, scaffolds, and biofactors for bone regeneration. The high level of need for bone grafts has caused researchers and surgeons to continue to develop biomaterials as an alternative option for restoring damaged bone tissue (Carpena, Min, & Lee, 2015; Rahman, 2015). This material is called alloplast or alloimplant. The material used must have the same characteristics as natural bone, namely it can come from non-metallic synthetic materials which can be obtained from ceramics (potassium phosphate), composites and polymers. One of the bioceramic materials that is often used in biomedical applications as a bone graft material is synthetic hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$.

Rosella application was carried out by injection into the area of bone damage and the extent of the bone damage area was analyzed one week after rosella application. It is hoped that with this application there will be inhibition of alveolar bone damage so that new therapies can be found for tooth relapse after orthodontic treatment and can have broad benefits from an economic and health perspective for the community (Jessylin, 2018).

METHOD

This research uses the MTT Assay method which was perform at the Prodia Stem Cell Laboratory in Indonesia. Researchers used 500 grams of red rosella flowers in dry form and hydroxyapatite granules + gelatin + HMPC 2%.

RESULT AND DISCUSSION

Result

This research uses osteoblast cells which play an important role in bone remodeling and preventing relapse. These cells were treated with hydroxyapatite-gelatin and rosella extract at concentrations of 15%, 20%, 25%, and 30%. Rosella flower extract is made by maceration and 96% ethanol solvent is added with a powder to ethanol ratio of 1:4. The purpose of administering this extract is to see the

toxicity of rosella flower extract in making bone graft scaffolds.

The cytotoxicity test was carried out using the MTT Assay method. In a 96-well microplate, osteoblast cells were treated. After 48 hours of incubation, MTT was added to the 96-well microplate at 100 µl/well. This method works by reducing active cell metabolism with the dehydrogenase enzyme which can produce purple formazan. The formation of purple formazan indicates the number of living cells. After being given MTT, the cells were incubated for 4 hours, which is the optimal time for formazan crystal formation. Then the Solubilization Solution is given.

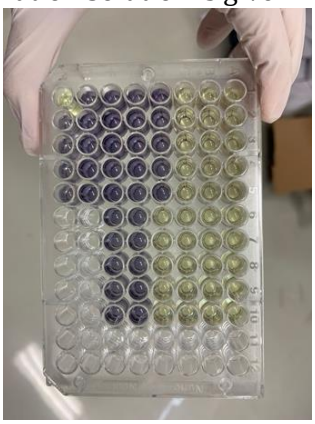
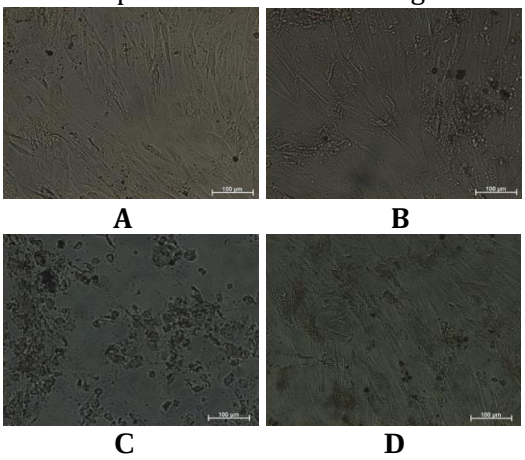


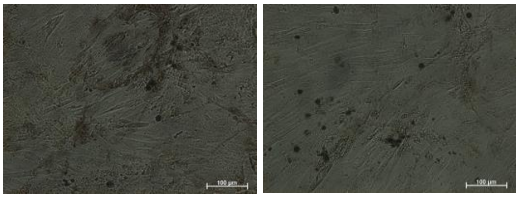
Figure 1. Condition of 96-well Microplate After Administration of MTT.

Observations of osteoblast cells after being given MTT were carried out using a microscope with the results in Figure 2.



A **B**

C **D**



E **F**

Figure 2. Observation of Osteoblast Cells using a Microscope. A. Control 1; B. Control 2; C. Treatment 15%; D. Treatment 20%; E. Treatment 25%; F. Treatment 30%.

	Kelompok	1	2	3	4	5	Average
1	HA + Gelatin + Ekstrak rosella 30%	0.401	0.361	0.389	0.382	0.362	0.379
2	HA + Gelatin + Ekstrak rosella 25%	0.354	0.400	0.384	0.400	0.368	0.3812
3	HA + Gelatin + Ekstrak rosella 20%	0.339	0.360	0.366	0.354	0.395	0.3628
4	HA + Gelatin + Ekstrak rosella 15%	0.220	0.274	0.354	0.307	0.311	0.293
5	HA + Gelatin	0.334	0.341	0.331	0.333	0.358	0.3394
6	Cell only (Negative Control)	0.337	0.313	0.317	0.311	0.337	0.323

Figure 3. MTT Assay Test Results.

From the absorbance results, cell viability was then calculated using the cell viability formula and the results obtained were that the 15% concentration had the lowest value of 182.92%, the 20% concentration was 1.3393%, the 25% concentration was 354.87%, and the 30% concentration was 264.66%.

The function of calculating cell viability is to carry out linear regression analysis using Microsoft Excel and the linear regression analysis graph of log concentration on cell viability is presented in Figure 4.

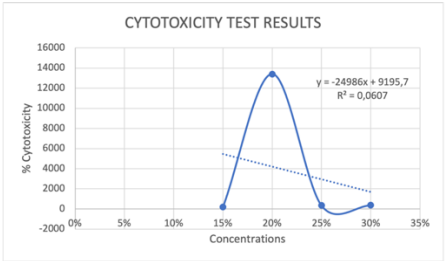


Figure 4. Line Equation for Determining IC_{50} of Rosella Extract on Osteoblast Cells.

After calculating cell viability, normality was then tested using the Shapiro-Wilk test using the SPSS application. The data distribution results from concentrations of 15%, 20%, 25%, and 30% in table 1 have a P value > 0.05 which can be said that each concentration has normally distributed data.

Table 1. Significance Test Results with Shapiro-Wilk.

No	Concentrations	Results
1	15%	0,855
2	20%	0,718
3	25%	0,412
4	30%	0,459

SCAFFOLD REQUIREMENTS

A dental material or substance that will be used as a treatment must meet biocompatible requirements. The scaffold in bone tissue engineering should ideally be osteoconductive and osteoinductive. Osteoconductivity relates to its ability to support attachment and formation and deposition of bone matrix. Osteoinductive is intended to template osteogenic precursor cells to differentiate into cells capable of forming bone. Thus, the scaffold model must be able to recruit progenitor or stem cells for healing bone areas where they will ultimately differentiate into extracellular matrix secreted by osteoblasts (Indahyani, 2008). Scaffolds are designed to play a role in supporting cell-biomaterial interactions, cell adhesion and extracellular matrix deposition, providing a supply of gas, nutrients and regulatory factors so that proliferation, differentiation, cell maturation can occur, delivery tools for cells, facilitating the distribution of cells in tissue that will grow, providing room for vascularization, new tissue formation and remodeling (Herda & Puspitasari, 2016). Hibiscus sabdariffa L. or Rosella is a herbal plant that is known to contain flavonoids which have the potential to treat alveolar bone damage because they have anti-inflammatory and antibacterial effects. Rosella also contains *delphinidin-3-sambubioside* which can reduce inflammatory mediators thereby inhibiting osteoclastogenesis (Erik Idrus, 2020). Rosella also contains flavonoids which can inhibit bone resorption by suppressing osteoclast activity. Flavonoids can speed healing by increasing cell proliferation and collagen synthesis at the wound site (Da-Costa-Rocha, Bonnlaender, Sievers, Pischel, & Heinrich, 2014; Majeed & Ghani, 2018). A material can be said to be biocompatible if the material does not cause irritation to living tissue, does not cause a toxic response, is free from allergens, and does not have carcinogenic potential. To determine the biocompatibility of a substance, a biocompatibility test or cytotoxicity test can be carried out to determine the effect of concentration on a substance, namely by carrying out the MTT Assay test (Nararya, 2015). The aim of this research is to review various materials commonly used to manufacture scaffolds for bone tissue engineering applications. Scaffolds that enter the oral environment must be degraded when the damaged tissue has been regenerated. The biocompatibility characteristic of implant materials means that the biomaterial must be easily accepted by the immune system, so that the body does not reject it and accepts it as part of the host body.

FLAVONOID CONTENT IN ROSELLA FOR BONE REPAIR

The results of this study show that the increase in the number of osteoblast cells that occurs after the MTT test at a concentration of 25% shows the activity of flavonoids in accelerating the healing process. In Bhaskar and Nithya's research, the wound healing potential of ethanolic extract of Hibiscus rosa sinensis flowers in mice and found that this extract could increase cell proliferation and wound collagen synthesis which was associated with the high content of flavonoids which are also contained in Hibiscus sabdariffa L. Other research conducted by Majeed et al., (2018) regarding the application of flavonoid extract from rosella for defects in rat legs shows that flavonoids have potential activity in the process of bone defects by suppressing osteoclast activity and increasing osteoblast formation (Majeed & Ghani, 2018).

Flavonoids can stimulate the proliferation of osteoblast cells into osteocytes by increasing the activity of estrogen receptors and increasing the growth of TGF-1 so that it can stimulate the proliferation of osteoblast cells (Sherman Salim & Mefina Kuntjoro, 2015). Apart from

flavonoids, rosella extract also contains tannin and quercetin compounds which also have an effect on bone repair. Sukmana et al., (2017) stated that tannin compounds can reduce IL-1β expression and increase BMP-2 during the bone remodeling process. Both of these things greatly influence the bone density process, IL-1β plays a role in inflammatory cells at the bone resorption stage, while BMP-2 induces the formation of osteoblast cells (Sukmana, Budhy, & Ardani, 2017).

Research by Sok Wong et al., (2020) shows that the quercetin compound can increase new bone formation and reports that this compound is osteogenic or has great potential in increasing bone formation. The alveolar bone remodeling process has an important role in preventing relapse after orthodontic treatment, as in research by Franzen et al., in their research with animals. Bone remodeling is controlled by osteoclast cells that absorb bone and osteoblast cells that produce new bone. Thus, relapse after orthodontic treatment can be prevented with activities that can inhibit bone resorption and stimulate bone formation (Arowoogun et al., 2021).

CONCLUSION

Based on the research above, rosella flower extract as an ingredient in making bone graft scaffolds is not toxic to osteoblast cells because cell viability calculations show that the percentage of living cells is > 50% which can be said that a sample is not toxic to osteoblast cells and can have potential in bone repair in preventing the occurrence of tooth relapse after orthodontic treatment. However, it is necessary to carry out pre-clinical tests on animals to determine the effective dose and side effects of using rosella flower extract as an ingredient in making scaffolds.

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