Synthetic Coral Scaffold and Platelet Rich Plasma for Bone Remodelling after Tooth Extraction

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ABSTRACT

Background: *The human body can self-repair. However, if the damage occurs excessively or reaches a critical defect, it requires material substitution to restore its shape and function. Tissue engineering is developed based on the principle of reconstructing damaged tissues due to critical defects to restore, maintain, and repair damaged tissues or organs by applying three main components: scaffolds, signaling molecules, and cells. This study aims to investigate the ability of synthetic coral scaffold incorporated with Platelet Rich Plasma in bone remodeling after tooth extraction.*
Method: *This study was conducted as in vivo experimental laboratory study with a post-test control group design. The 48 male Rattus norvegicus rats were divided into 4 treatment groups. Firstly, the rats' teeth were extracted, then, on the tooth extraction area, it was treated with povidone-iodine (control group), curaspon, scaffold only, and scaffold-incorporated Platelet Rich Plasma.*
Result: *The results of the Kruskall-Wallis test showed no significant difference ($p > 0.05$) between the four treatment groups. article*
Conclusion: *The PRP-incorporated synthetic coral scaffold had the highest score of collagen formation density and significantly accelerated collagen formation in the bone remodeling process compared to other treatment materials.*

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INTRODUCTION

Injuries that occur in the human body will cause tissue damage. Furthermore, regeneration processes will occur where the body carries out the healing process to function again. Wound healing can occur depending on the tissue type in the wound area for tissue regeneration. The connective tissue will be formed if tissue regeneration cannot be carried out. For minor injuries or damage that is not too big, the body can make repairs (self-repair), and a healing process occurs so that the tissue can return to its original shape and carry out its functions normally, called regeneration. New cells replace damaged or dead cells to restore tissue to its original state. However, if the damage experienced is too large and involves a large area called a critical defect, then the body cannot regenerate perfectly, and scar tissue will appear. In this case, regenerating large tissue damage requires material substitution to help the regeneration and functioning of the tissue again.[1] Tissue engineering combines several fields of science that aim to restore, maintain, and repair damaged tissues or organs. Tissue engineering is developed to reconstruct damaged tissue by applying three main components: scaffolds, signaling molecules, and cells. These three components can be used separately or together.[2] Cells are an important element of tissue engineering. Cells need a suitable environment to carry out their functions. The scaffold provides an environment for cells; for this reason,, the cells used in tissue engineering can be cells incorporated into the scaffold, namely cells that come from outside the network or can also rely on cell sources from within the tissue.[3]

Scaffold is a component of tissue engineering that provides structural support for cells to carry out attachment, proliferation, and differentiation.[4,5] For example, a scaffold made from calcium carbonate with a well-connected porous structure can be a potential material for bone tissue engineering applications.[6] Gelatin is a heterogeneous mixture of polypeptides obtained by partial hydrolysis of collagen in connective tissue, skin and bones in animals. Gelatin can also be used to manufacture scaffolds as it is biocompatible, biodegradable, and has low antigenicity.[7,8] Various kinds of gelatin sources can be obtained; 45% came from pig skin, 29.4% came from cow skin, 23.1% from bone, and 1.5% from other sources.[9] Indonesia has a majority Muslim population, so it is necessary to pay attention to the source of the gelatin used so that all groups can use it. Gelatin from cows is a source that can meet this need.[10]

The success of tissue engineering is also influenced by signal molecules that can be obtained from growth factors. Signal molecules have a role in stimulating cells in the tissue to proliferate and differentiate into target cells, in this case, bone cells. This study used platelet-rich plasma (PRP) as a signaling molecule. PRP is the concentration of platelets to produce an extracellular matrix.[11] Scaffolds are also expected to be incorporated with signal molecules.[12] The properties and characteristics of the scaffold are considered when designing scaffolds for use in tissue engineering, including biocompatibility, biodegradability, mechanical properties, and high porosity to be adequate for the distribution of signal molecules and nutrients into cells. [4,13]

In recent years, sea coral has become a material often used as a scaffold as it is easy to process and contains calcium carbonate, which is effective as the main ingredient for bone replacement. However, sea coral is included in a protected ecosystem, so to protect the habitat of the coral, an alternative was chosen in the form of synthetic coral, which can still function as a scaffold for the bone regeneration process.[7] The scaffold that resembles coral has been developed with calcium carbonate (CaCO3) and cow gelatin as an alternative material to replace sea coral. Calcium carbonate is a synthetic biomaterial that can be used as a base material to

manufacture scaffolds. Calcium carbonate has high biocompatibility, bioactivity, and osteoconductivity properties, so it is suitable for the implementation and regeneration of bone,[2,14]

Signal molecules needed in tissue engineering can use Platelet Rich Plasma (PRP). PRP is a rich source of cytokines, interleukin 8, and growth factors. It contains various growth factors, including platelet-derived growth factor (PDGF), transforming growth factor (TGF), vascular endothelial growth factor (VEGF), and insulin-like growth factor (IGF).[15] PRP can also accelerate the process of bone formation and maturation. It can happen as several growth factors produced by PRP, such as platelet-derived growth factor (PDGF), transforming growth factor (TGF), and insulin-like growth factor (IGF), when released, will trigger bone formation.[16]

Bone remodeling is a process that occurs continuously throughout life to repair bone damage, prevent bone accumulation, and maintain mineral homeostasis by releasing calcium and phosphorus reserves. When a critical defect occurs, remodeling plays a role in maintaining and protecting the body's organs and is an important physiological process in increasing bone strength.[17,18] Likewise, after tooth extraction, bone remodeling is required after damage to the supporting bone of the extracted tooth.[19] This study aims to determine the effect of applying synthetic coral scaffolds incorporated with PRP in bone remodeling after tooth extraction.

RESEARCH METHOD

This study is experimental laboratory research with a post-test control group research design. This research was conducted at the Experimental Animal Laboratory of the Faculty of Medicine and Health Sciences UMY, the Integrated Research Laboratory of the Faculty of Dentistry UGM, and the Clinical Pathology Laboratory of AMC Muhammadiyah Hospital.

Preparation of experimental animals and Platelet Rich Plasma

White rats (Rattus norvegicus) Wistar strain, male, 2-3 months old, and weighing 150-200 grams were prepared. 48 rats were divided into 4 groups. Rats, after maxillary incisor extraction, were divided into 4 treatments, namely given povidone-iodine (group I), given with curaspon hemostatic agent (group II), given synthetic coral scaffold incorporating PRF (group III), and given synthetic coral scaffold without PRF incorporation (group IV). Each group consisted of 12 rats which 3 rats were decapitated on days 7, 14, 21 and 28. Platelet Rich Plasma was isolated from whole blood and centrifuged with a refrigerator centrifuge at 3000 rpm for 10 minutes until the red blood cells, PRP and serum were separated. PRP was taken and ready to be incorporated.

Synthetic coral scaffold and PRP incorporation

The scaffolds were made from type B gelatin (Nitta Co., Osaka, Japan) and calcium carbonate calcite ($CaCO_3$) (Wako, Osaka, Japan) with a ratio of 5:5 at a concentration of 10% w/v in distilled water. Sodium citrate (Sigma-Aldrich, Germany) was prepared as a dispersant. Calcium carbonate was added and stirred in the gelatin until a homogeneous suspension solution was obtained. The suspension was then molded to prepare sheet-shaped scaffolds with a thickness of 0.3 mm. The scaffolds were frozen at -20℃ for 24 hours, followed by freeze drying for 24 hours. After that, the scaffolds were cross-linked using the dehydrothermal method using

a vacuum oven (Memmert, USA). The scaffolds were prepared in sheet form with a 6 mm diameter using a biopsy punch (Figure 1A). Before incorporation, the scaffold sheets were sterilized using ethylene oxide gas at 36℃. The finished PRP was prepared, incorporated into the scaffold group using the drip method of as much as 50 µl, and waited for 15 minutes to obtain maximum absorption into the scaffold (Figure 1B).

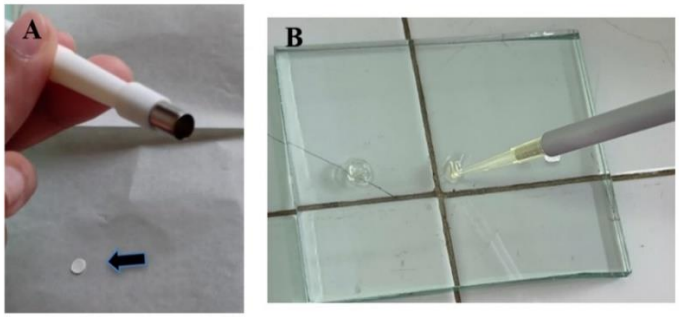

Figure 1A. Scaffold preparation for application (arrow – scaffold) B. PRP incorporation into the scaffold

Tooth extraction and material application

Extraction was performed on the rats' maxillary incisors by anesthetizing the rats using a combination of ketamine (40mg/kg) and xylazine (5mg/kg). After that, the extraction socket was irrigated using distilled water to remove debris or remnants of tooth extraction. Furthermore, povidone-iodine was applied to group I, hemostatic agent curaspon (II), synthetic coral scaffold incorporation of PRP (III), and synthetic coral scaffold only (IV) (Figure 2). White rats were kept in clean cages and given food and water. Routine checks were carried out to ensure the condition of the rats.

Rats were sacrificed on days 7, 14, 21, and 28 to observe the bone remodeling after tooth extraction histologically. The maxillary part of the extraction section was excised and decalcified using EDTA. Paraffin embedding and Hematoxylin Eosin staining were performed to observe bone remodeling.

Figure 2A. Maxillary tooth extraction; B. Application of scaffold on the post-extraction area

Data collection and statistical analysis

Histological object glass stained with HE was then observed using a light microscope with 40x magnification (Figure 3). Retrieval of bone remodeling data using scoring with collagen parameters was shown in Table 1.

Table 1. Scoring of bone remodeling assessment

Score	Information
0	Shows no collagen density
1	Shows minimal collagen tissue
2	Shows light collagen tissue
3	Shows moderate collagen tissue
4	Shows a density of collagen tissue.

Data were analyzed with SPSS using the Shapiro-Wilk test, and the homogeneity test was carried out with the Levene Test. Furthermore, a parametric one way ANOVA test was performed with a 95% confidence level ($\alpha = 0.050$) and continued with the LSD Post Hoc (Least Significant Difference) test to determine significant differences between groups.

RESULTS

Bone remodeling is a physiological process that occurs throughout life and plays an important role in renewing the skeleton. This process maintains and enhances bone strength by replacing the micro-damaged primary and mature bone with new bone and plays a role in maintaining calcium homeostasis. In the process of bone remodeling, there is a phase of collagen formation. In normal tissue, collagen synthesis and degradation processes occur, followed by the replacement of new tissue fibers required for the remodeling process. Type 1 collagen is involved in mechanical functions to provide elasticity and form tissue components. Collagen is important in forming bone strength to absorb energy.[20] Figure 3 presented the density of collagen fibres 14th and 21st days between 4 treatment group. The PRP incorporation is served scanning electron microscope, that showed the platelets are filled the pores of scaffold. And the results of bone remodeling scoring after tooth extraction with collagen parameters can be seen in Table 2.

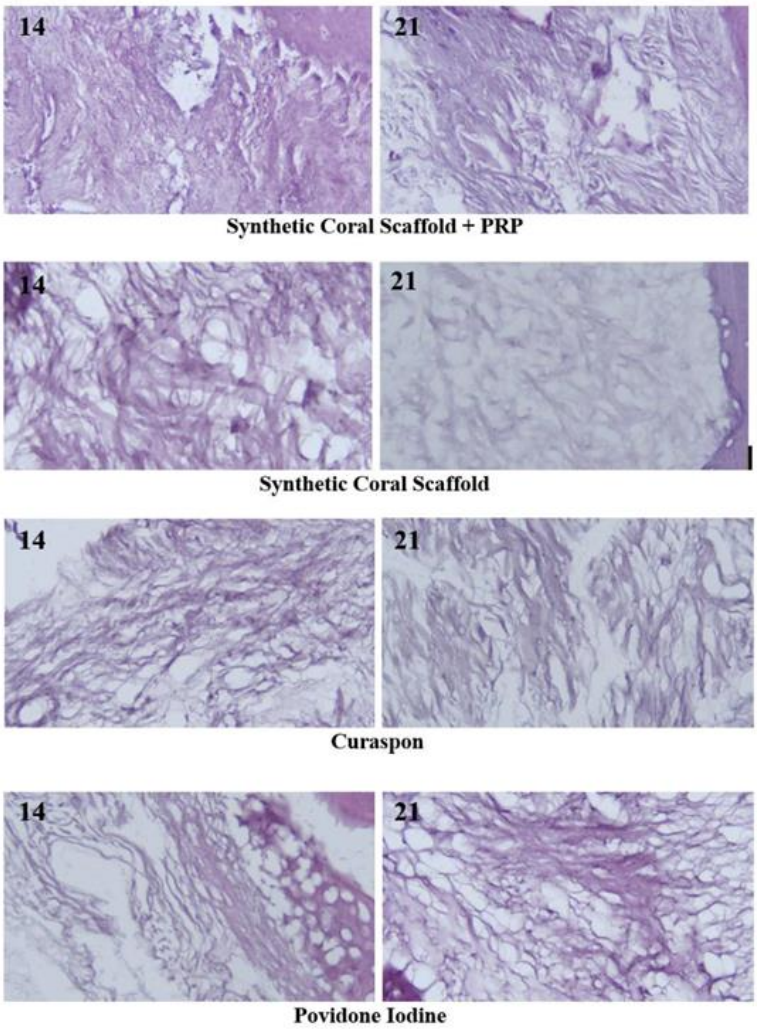

Figure 3. Density of collagen in the bone remodeling process of 4 treatment groups on day 14 and 21.

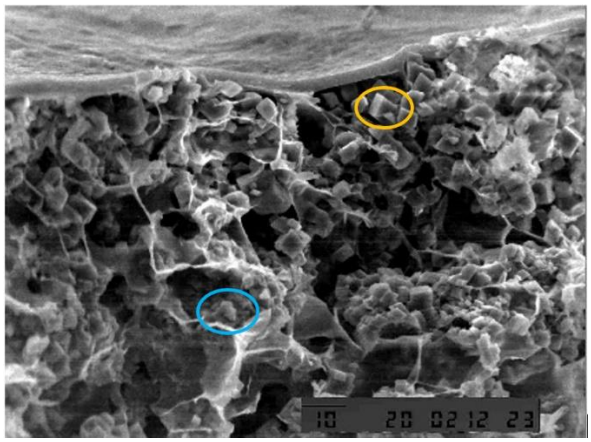

Figure 4. Scanning electron microscope of synthetic coral scaffold incorporated PRP. The pores with interconnection between pores are filled platelets from PRP, yellow circle: calcium carbonate, blue circle: platelet.

Table 2. Post-extraction bone remodeling scoring results

Observation Day	Scaffold + PRP	Scaffold Only	Curaspon	Povidone Iodine
7th day	2	1	1	0
	2	1	1	1
	2	1	1	0
14th day	3	2	1	1
	2	1	1	2
	3	1	1	1
21st day	2	1	2	2
	3	2	2	3
	3	2	3	2
28th day	4	3	3	4
	4	3	4	4
	3	3	4	3

Statistical analysis using Kruskal Wallis is presented in Table 3. The Kruskall Wallis test in Table 3 shows no significant difference in the bone remodeling scoring results between the four treatment groups. However, PRP-incorporated Synthetic Coral Scaffolds had the highest score for collagen formation. Therefore, it can be assumed that the PRP-incorporated Synthetic Coral Scaffold is more influential in accelerating collagen formation in the bone remodeling process than other treatments.

Table 3. Kruskal Wallis test results

Treatment	N	Mean	Mean Rank
Scaffold Only	12	1.75	20.13
Curaspon	12	2.00	22.67
Povidone Iodine	12	1.92	22.42
Scaffold + PRP	12	2.75*	32.79*
Total	48	2.10	

	Score
Chi-Square	6.277
df	3
asymp. Sig.	.099*

DISCUSSION

After extraction, bleeding occurs in the tooth socket, and the blood clot closes the socket. The inflammatory process stimulates increasing pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin-1β (IL-1β), and osteoclasts. If the number of osteoclasts increases, alveolar bone resorption will occur.[19] The healing process in the socket after tooth extraction will occur the formation of woven bone, then remodeling will occur in the form of repair of the defect. In the first 4 weeks after tooth extraction, a fibrinous collection of blood clots forms, which is replaced by fibrous tissue and blood vessels. Between 4 to 8 weeks after extraction, the osteogenic tissue will proliferate and form trabecular bone, followed by bone maturation (Devlin & Sloan, 2002).[21,22]

The Curaspon treatment group, or the positive control group, had the second-highest score in increasing collagen formation during bone remodelings. Curaspon is a gelatin sponge hemostat agent with absorbable properties and plays a role in wound healing. A gelatin sponge can accelerate the blood coagulation

process, so tissue proliferation can occur immediately around the injured area. Curaspon can form bone on the 28th day, where the bone remodeling process takes place on the 21st day and continues for about 1 year.[23]

PRP are the concentrated of platelet. Platelets perform three distinct functions in hemostasis namely providing structure and support for blood clotting, releasing additional coagulation factors to support the coagulation cascade, and releasing von Willebrand (vWF) and fibrin to augment blood clot matrix formation. Disc-shaped platelets in circulation. Electron microscopy analysis of platelets shows some internal structure. Firstly, platelets have two main categories, namely granules and solid granules. Granules number approximately 50 to 80 per platelet. The granules contain proteins such as fibrinogen, factor V, factor XIII, vWF, and plasminogen. Solid granules number 5-7 per platelet and contain small molecules such as ADP, ATP, Ca21, and serotonin.[24] Platelets contain solid granules, alpha grains, lysosomes, and after activation can secrete growth factors, adhesion molecules, and chemokines which will interact with the local environment to promote cell differentiation, proliferation, and regeneration.[25]

PRP, with the addition of a bone-graft scaffold, is beneficial in increasing the effectiveness of platelet concentrates as an innovation in alveolar bone regeneration therapy.[26] The platelet incorporated in pores of scaffold and attached on the wall of pores (Figure 4.) The platelets will be released simultaneously with the degradation process of the scaffold.[3] PRP as platelet concentrate, which contains growth factors such as PDGF, TGF-β, VEGF, and IGF, will stimulate matrix metalloproteinase, which plays a role in collagen production. These growth factors promote cell proliferation and differentiation as well as osteoprogenitor migration in areas of alveolar bone damage.[22] The collagen density of synthetic coral scaffold incorporated PRP show It was also reported that the combination of the silk-fibroin scaffold of silkworm cocoons with platelet concentrate has an effect on accelerating the process of bone formation and maintaining alveolar bone volume.[26] PRP can release various growth factors and promote bone healing in oral and maxillofacial surgery. PRP has an important role as a hemostatic in physiological processes and wound healing due to its high content of growth factors.[27,28] In line with these studies, synthetic coral scaffold with PRP incorporation can speed up the post-tooth extraction bone remodeling process.

CONCLUSION

The PRP-incorporated synthetic coral scaffold had the highest score of collagen formation density and significantly accelerated collagen formation in the bone remodeling process compared to other treatment materials.

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