Degradation, swelling profile, and gel fraction of synthetic coral scaffold incorporated PRP or PRF

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Abstract. Platelet rich plasma (PRP) and platelet rich fibrin (PRF) are sourced from human blood and have role in bone recovery. Both of them could be incorporated into scaffold for bone regeneration. Synthetic coral scaffold is a natural mimicking of sea coral designed for bone regeneration. It should have bio degradability and bio absorbability that influence new bone formation process. The purpose of this study is to investigate the degradation, swelling profile and gel fraction of synthetic coral scaffold incorporated with PRP or PRF. Synthetic coral scaffold consists of gelatin, calcium carbonate, and sodium nitrate as dispersant agent. It is divided into 3 groups, incorporated PRP, PRF, and no-incorporation. Scaffold was soaked in phosphate buffer saline (PBS) and incubated in 37° C for 24 hours. Scaffold weight was measured in every 30 minutes to observe the swelling profile and gel fraction. Degradation profile was observed after 1, 3, 6, 24, 48, 72, and 96 hours of soaking. Acceleration of degradation was measured after soaking with HCl 1N for 1, 3, 6, 24, 48, 72, 96 hours until the scaffold ran out. The result shows significant differences among PRP, PRF, and control group. PRP incorporation has slow degradation, good swelling and higher gel fraction that presents the strengthening of scaffold structure.

1. Introduction

Bone as supporting tissue forms structures and protects vital organs of the body. Bone can be damaged due to trauma or malignancy. Bone damage must be treated immediately to prevent local or systemic damage [1]. Various alternative treatments can be done to repair bone damage, such as bone graft to accelerate bone damage recovery. Bone graft is a bone tissue substitution derived from autologus, artificial, synthetic, or natural substitute material. Bone grafting may be done because the bone has ability to fully regenerate in adequate space. As bones that grow naturally, bone tissue generally replaces all bone grafts to produce an area containing new bone [2].

Tissue engineering aims to regenerate tissue and create biological substitutes for deformed or damaged tissue and organs that require cells. Tissue engineering technology is currently developed rapidly to facilitate tissue regeneration as needed [3]. Tissue engineering technology involves three factors that greatly influence the success of tissue reconstruction. The three main factors are signals, cells, and scaffolding [4].
The development of tissue engineering has been very advanced especially in the field of dentistry. The principle application of tissue engineering has expanded in various branches of dentistry, such as periodontics, maxillofacial surgery, and dental implants. Signal is an important component that can be obtained from growth factor. Growth factor can be obtained from PRP (Platelet Rich Plasma) and PRF [3]. PRP is the result of separating whole blood which is centrifuged to a certain level and in the form of a plasma layer consisting of platelet rich and poor plasma, and red blood cells. PRP contains many growth factors and other components that are very helpful for healing and regeneration process [2]. The difficulties encountered in making PRP cause evolution and simplification that result in PRF (Platelet Rich Fibrin), which has all the useful components for recovery and healing [5]. PRF contains more white blood cells that are important for wound healing process [6], and, it is the result of PRP development, namely the second generation of PRP. The process of PRF manufacture is easier and simpler than PRP because no biochemical ingredients are added, such as thrombin and anticoagulants [7]. PRF is also an autologous agent similar to PRP because it derives from the patient's own body [8]. Platelet rich fibrin are able to support bone regeneration, because it contains a lot of platelets, fibrin matrix and growth factor [9].

Besides acting as signals, PRP and PRF also have property as scaffold themselves because PRP and PRF contain fibrin. Fibrin can be used as a scaffold material because fibrin is an autologous scaffold that does not cause toxic or excessive immune reactions. It derives from the individual itself. The mechanical properties of fibrin are limited, and thus the utilization is limited. However, fibrin can be combined with other scaffold materials so that it can strengthen the structure of the scaffold itself and the time of growth factor release in accordance with the scaffold degradation [10]. PRF is a fibrin matrix containing cytokines, namely, platelets that contain growth factors and can function as resorbing membranes [9].

Scaffold has an important role in the success of tissue engineering. Various biological properties must be present in scaffolding, such as biocompatibility, biodegradation, high strength and high porosity [11]. Biocompatible means the ability of scaffold of being received by the body and degradable when the formation of new cells has been achieved. The non-degradable scaffold inside the tissue can initiate some infections in the body. The ideal velocity of scaffold degradation is equal to the velocity of tissue formation [12]. The mechanical properties of the scaffold are influenced by several factors such as original rigidity of polymer chains, types of molecular crosslinking, crosslinking and swelling solidities as the result of hydrophilic or hydrophobic balance. One of the design parameters in tissue engineering that determines its success is the mechanical properties of the scaffold. It is intended that the scaffold must be able to provide and maintain space for developing cells. In addition, the scaffold mechanical properties also influence genes adhesion and expression from the cells [10]. The biocompatible properties are influenced by the hydrophilic characteristic from constituent material of the scaffold. The good biocompatible properties of a scaffold can be shown by the ability to absorb liquid medium by the scaffold membrane without dissolving it in the liquid medium or referred to as swelling ability. This relates to the swelling profile of a scaffold which has an important role in supporting cell nutrition distribution during tissue regeneration process [13]. The swelling profile on scaffold is one of its characteristics. The swelling profile represents the process that occurs in scaffold when swelling after the fluid enters the scaffold and after the maximum swelling is obtained, it will burst [14]. Scaffold swelling ability will work optimally over large areas [15].

Synthetic coral scaffold is designed to resemble natural sea coral, has porosity, and can provide space for cells to attach and grow [16]. It also has a degradation profile that meets the criteria as a scaffold for bone tissue regeneration. This can also be incorporated with PRP for the operation of tissue engineering system [17]. The additional platelet concentrated into the scaffold material in bone regeneration serves to stimulate the proliferation and differentiation of osteoblast cells and osteoprogenitor around the bone damage area. PRP and PRF have poor mechanical properties, low stability and very fast degrees of
degradation. Mastication also disrupts bone formation process. The addition of scaffold material is expected to provide mechanical strength and maintain the stability of bone graft material during bone recovery process. The time of scaffold degradation becomes quite long so that the recovery phase occurs slowly and can run more optimally [18].

2. Material and method
2.1. Scaffold preparation
Synthetic coral scaffold was developed from gelatin and calcium carbonate with a ratio of gelatin: calcium carbonate 5:5 and 10% gelatin as a control. Sodium citrate was used as a dispersant in a small composition. The solution was molded into a thick film-like form, and freeze dried afterward. After the manufacturing process, physical crosslinking was carried out to strengthen the structure [16].

2.2. Preparation of PRP and PRF
PRP and PRF were obtained from 5 human whole blood donors who had agreed to ethical clearances. Blood was collected in vacutainer with EDTA and then centrifuged for 2 times. First with a velocity of 1200 rpm for 10 minutes, buffy coat was taken, transferred to a new vacutainer, and then centrifuged at 3500 rpm for 10 minutes to obtain PRP.

As for the PRF isolation, whole blood was put into plain vacutainer (without anticoagulants) and was immediately centrifuged to avoid blood clots. It was centrifuged for 10 minutes at a velocity of 1600 rpm until it formed 3 layers, namely, PPP, fibrin clot (PRF) and red blood cells. The fibrin clot or PRF layer was placed in the center of the tube. Fibrin clot was transferred to a new vacutainer and was put in the refrigerator for 24 hours.

2.3. Incorporation into synthetic coral scaffold
The incorporation of PRP and PRF into artificial synthetic coral was done by immersing the scaffold in each microtube which has been filled with PRP and PRF for 10 minutes until it was absorbed into the scaffold. Scaffold was removed from the microtube, and the remaining droplets of PRP and PRF were drained. The amount of PRP and PRF used for incorporation was 100 ul for swelling profile observation and 40 ul for profile degradation.

2.4. Swelling observation
After the incorporation of PRP or PRF, firstly, scaffold with incorporated PRP/PRF was weighed as $W_0$ wet. Each scaffold was put in a pot and filled with a 3 ml PBS solution until the scaffold is completely immersed. It was then incubated at 37 °C with a period of 30 minutes, 1, 2, 4, 6 and 24 hours [19]. Each period of time, each sample was lifted and drained with a filter and then wet scaffold was weighed ($W_w$).

The percentage of swelling in observing the swelling profile was obtained by formula:

\[
\text{Swelling} \, \% = \frac{W_w - W_0}{W_0} \times 100
\]

$W_0$ = the initial weight of the scaffold before immersion.
$W_w$ = the wet weight of the scaffold after immersion.

2.5. Degradation observation
Each scaffold used for degradation observations before incorporation of PRP / PRF weighed 10 mg. Three scaffold groups was immersed into each microtube which was filled with 1.3 ml PBS and then incubated at 37 °C for 1, 3, 6, 24, 48, 72 and 96 hours. At each time period, it was centrifuged at 3500 rpm for 2 minutes so that the scaffold was at the bottom of microtube. The supernatant was taken and measured its optical density with the UVmini-1240 SHIMADZU spectrophotometers (wavelength 280 nm). Microtube containing scaffold deposits were filled with PBS and incubated again. This was done repeatedly until the last measurement period.
The degradation acceleration was observed by replacing the immersing solution with 1N HCl and by measuring as before until the scaffold was perfectly degraded.

After getting the absorbance value for each time interval, scaffold degradation percentage then was calculated by the following formula: [20].

\[
\text{\% scaffold degradation} \times \text{period} = \frac{\text{X period absorbance value}}{100\% \text{ absorbance value}}
\]

3. Result
3.1. Swelling Profile
The time needed for incorporation was 10 minutes. The 0-minute period was the time for scaffold to dry before the incorporation of PRP or PRF and its immersion in PBS. The 10th minute period was the time for scaffold to immerse in PBS after incorporation for 10 minutes. This caused scaffold incorporated by PRP and PRF to gain significant weight compared to the control scaffold. The treatment of PRP or PRF incorporation shows the swelling process in the scaffold. The 30th minute time period, as shown in Figure 1, shows that the ratio of swelling profiles of PRP incorporated scaffold was higher compared to the swelling profile ratio of other scaffolding groups. After 30 minutes, the swelling profile in all groups decreased and in 24 hours it appeared to be a rather sharp decrease in swelling profile except the PRP incorporation group.

![Swelling profile](image)

**Figure 1.** Swelling profile

| Test of Homogeneity of Variety | Anova | Post Hoc |
|-------------------------------|-------|----------|
| Levene Statistic              | Mean Square | Sample | Sig. | Sig. |
| Sig.                          | Between Group | Within Group | PRP - PRF | .068 |
| Sig.                          | 1.531 | .294 | .013 | PRP - control | .036 |
| Mean Square                   | 128.251 | 13.071 | PRF - control | .858 |

**Table 1.** Gel fraction statistical analysis
Table 1 shows that the percentage of gel fraction in the PRP incorporation group with control group has a significant difference, whereas the difference is not significant in PRF group with PRP and PRF with the control group (p <0.05).

The highest percentage of gel fraction is the PRP incorporation group shown in Figure 2. This shows that this group has a structure and mechanical properties that are stronger than other of other scaffolding groups.

3.2. Degradation Profile
Based on Figure 3 scaffold with incorporation of PRP and control (without incorporation) is degraded longer than scaffold with incorporation of PRF. Scaffold with incorporation of PRF on acceleration degradation using 1N HCl solution is degraded 100% for the first time at the 216th hour compared to the other 2 groups.

4. Discussion
The PRP scaffold group and the PRF scaffold group had undergone a swelling process previously during the incorporation process in the first 10 minutes. It was 223.95% in the PRP scaffolding group and 203.54% in the PRF scaffold group. Meanwhile, the control scaffold group had not undergone a swelling process because of no addition of PRP or PRF. The first 30 minutes period, the three sample groups continued to swell so that the percentage of swelling still continued to increase. PRP scaffold increased as much as 38.41% and an increase of 37.71% for the PRF scaffold of the initial swelling profile ratio means that scaffold swelling capabilities were still increasing. This research is in accordance with the research conducted by Wahyudi and Nurwadji, which states that swelling ability or swelling profile ratio
will increase dramatically at the beginning of immersion time [21]. The increasing of the swelling profile ratio at the beginning plays a role in facilitating the process of cell migration and the formation of 3D structures. The next period, the scaffold swelling profile begins to decrease slowly with the PRP scaffold still at the highest position, but the value does not differ much from the PRF scaffold, while the control scaffold has the lowest swelling profile ratio. The next period, on the 4th and 6th hours of the scaffold swelling profile ratio tends to maintain its position until the 24th hour of immersion. According to Park et al., the swelling profile ratio will begin to decrease along with the increasing of immersion time, and after that the scaffold will experience an equilibrium phase [22]. Equilibrium phase is a phase in which the scaffold tends to maintain its weight. Based on research conducted by Zhang et al. [23], the swelling profile ratio of PVA/HA composite hydrogel is divided into 4 stages, namely increasing rapidly, decreasing, decreasing slowly and then balanced or entering the equilibrium phase, decreasing swelling ratio followed by increase in HA content in scaffold.

The significant difference between PRP and PRF is the presence of thrombin and calcium chloride which are anticogulant agents. This agent is important in helping in the fibrin polymerization process because there is no addition of thrombin and calcium chloride in PRF. The formation of fibrin matrix runs slowly, in contrast to PRP which is the addition of thrombin and calcium chloride the fibrin matrix formation process can run quickly. The fibrin matrix formation phase plays an important role in the formation of 3-dimensional structures of fibrin network [24]. Calcium ion concentration has a role in the change of fibrinogen to fibrin, could stabilize the structure of fibrinogen and accelerates fibrin formation by acting as a thrombin cofactor. The influence of the presence of thrombin in physical characters fibrin networks such as fiber diameter, mass/length, density, porosity and permeability can cause changes in cell adhesion and migration [25]. The presence of PRP activation by anticoagulants can help releasing the growth factors and cytokines from platelets and leukocytes which help stimulate the process of fibrinogen decomposition into fibrin fibers to form a network structure that can support the process of cell proliferation and differentiation. In addition, with the double centrifuge in the process of making PRP besides enriching platelet content, it is also beneficial to maintain the integrity of PRP so that it becomes more stable [26]. Research conducted by Rowe et al. mentions that differences in thrombin concentration added to fibrin hydrogel can affect the morphological structure of fibrin fibers in the 3D structure of it [27]. Whereas, low thrombin concentrations will increase bundle size fibrin so that the pores of fibrin hydrogel are larger and manage to increase cell proliferation and differentiation. It also can affect the structure of mechanical strength.

After 24 hours of immersion, the scaffold will undergo a process of breaking the structure or the so-called gel fraction process. Gel fraction was measured by drying the scaffold which is not soluble in PBS at a temperature of 500 in the oven and weighed every 24 hours until the weight is stable. The gel fraction process begins with a decrease in the mechanical properties of the scaffold which is characterized by a decrease in the weight of the scaffold. There is a difference in the gel fraction value of the scaffold group incorporated with PRP, a scaffold incorporated with PRF and control scaffold. According to a research by Park et al. [22], it is stated that the percentage of gel fraction would increase along the increasing time period and decrease in the higher PEG concentration in the PVA based Hydrogel scaffold. In addition, the gel fraction value is proportional to the tensile / mechanical strength of the scaffold. The formula used to calculate the percentage of gel fraction shows that the percentage of gel fraction is proportional to the final dry weight of the scaffold. It means the higher the percentage of gel fraction, the less weight the scaffold dissolves during the immersion process which shows the mechanical strength of the scaffold structure [28]. The results shows that among the percentage of gel fraction between 3 groups, PRP has the highest percentage of gel fraction which is indicated by the percentage of scaffold incorporated with PRP that is higher than the scaffold incorporated with PRF and control scaffolding. This shows that the scaffold incorporated with PRP has stronger mechanical strength compared to the scaffold that is incorporated with the PRF and control scaffolding.
The velocity of scaffold degradation must be in accordance with the velocity of tissue formation [29]. The degradation process is part of the release of molecules signal. The slower the process of scaffold degradation, the slower the molecules signal release to the location of bone reconstruction [30]. In line with the research that has been done, it is assumed that PRP releases the molecule signal more slowly than the PRF so that it has a slower degradation process. PRP is an autologous plasma that has a platelet concentration up to 1,000,000 / µl. The normal number of platelets in the blood is 150,000 / µl to 350,000 / µl. PRP has many growth factors which one of its functions is to stimulate collagen synthesis and to accelerate homeostasis responses in injuries so as to stimulate bone regeneration and wound healing processes [31]. PRP is a platelet-rich plasma derived from autologous or derived from the same body that is placed in the plasma and contains many growth factors. Growth factors found in PRP are PDGFαα, PDGFββ, PDGFαβ, TGF-β, TGF-β2, VEGF, and EGF [32]. Matsui and Tabata, states that platelet activation occurs when in contact with gelatin which can release various growth factors in PRP [3]. PRP contains a lot of platelets. PRP activation by thrombin or CaCl2 platelets releases α-granules and hydrogel which contain many growth factors including TGF-β [34]. TGF-β contained in PRP can increase collagen production and matrix minerals [31].

The combination of scaffold and PRP has several advantages. First, the porous structure of scaffold can stabilize the formation of fibrin network before PRP is activated by forming a 3D environment for growing and differentiating cells. Second, it controls and extends in releasing several growth factors, including TGF-β [33]. Based on the discussion, it can be assumed that one of the factors which slows down the release of growth factors in PRP is due to the porous structure of the scaffold. Anticoagulants are substances that can prevent blood clots by binding the calcium or by inhibiting thrombin formation to convert fibrinogen to fibrin in the clotting process [34]. PRP formation requires anticoagulants so that in line with the discussion. It is assumed that more calcium is found in the incorporation of PRP than in the incorporation of PRF. PRP requires fibrinogen, thrombin and calcium to form the fibrin network so that the growth factors derived from platelets can be released. The second step in PRP preparation results in activating platelets and fibrin network formation. This occurrence is similar to the natural clotting process where fibrinogen is broken down by thrombin and it is responsible for the hemostasis process. The breakdown of fibrinogen will produce a three-dimensional fibrin network. Calcium can stabilize fibrinogen structures, accelerate fibrin formation by acting as a thrombin co-factor, which is able to protect fibrinogen degradation. It can also inhibit and prolong the polymerization process of fibrin fibers. Fibrin networks are influenced by the fibrin polymerization reaction [25]. In this study, researcher used the artificial coral scaffold which contains calcium and anticoagulants. They can bind calcium, so, it can be assumed that the amount of calcium derived from artificial coral scaffolds and those bound by anticoagulants can increase fibrin network formation, stabilize fibrinogen structure and slow down the fibrinogen degradation. Overall, it can slow down the process of scaffold degradation.

Fibrin fibers produced from PRP are thinner and denser[35]. It is in line with the research of Perez et al., which states that thinner fibrin fibers can slow down clotting time [25]. It can be estimated that the increase in mechanical strength of the scaffold incorporated with PRP occurs because calcium contained in PRP and bound by anticoagulants and calcium contained in artificial coral scaffold can strengthen the structure by forming fibrin network and by slowing down the degradation process of fibrinogen. It causes PRP incorporated scaffolds becomes slower in degradation.

5. Conclusion
There was significant difference among PRP, PRF, and control group. PRP incorporation has good swelling profile, slow degradation profile, and higher gel fraction that presents strengthening of scaffold structure.
6. References

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