

Efficacy of Incorporation Platelet Rich Plasma into Gelatine Hydrogel Scaffold between Impregnated and Drop Method

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Abstract. Tissue Engineering which involve three main component such as scaffold, platelet-rich plasma (PRP) and cells is expected to support in bone regeneration. Gelatin hidrogel scaffold is planted have a function as cell environment and PRP provide growth factor to support differentiation of cells. The success of tissue engineering is affected by number of PRP which is contained in scaffold. The purpose of this study is to compare the incorporation process between impregnated and drop method to gelatin hidrogel scaffold. PRP was prepared from three donors of whole blood, and twice sentrifugation by 450 rcf for 5 minutes and 1500 rcf for 7 minutes. PRP was incorporated into 3 gelatin hidrogel scaffolds for each methods. The remnant of PRP which didn't incorporate were calculated the number of platelet with giemsa staining. Platelet which loaded were the reduction result of number platelet before incorporate with platelet remnant. Data of the result were analyzed using independent sample t test. Result show the significant was 0.262 ($p > 0.05$) there's no significane different between impregnated and drop method for incorporating PRP into gelatin hidrogel scaffold. The number of platelet which incorporated in gelatin hidrogel scaffold were effected by characteristic of scaffold such as structure, interface adherence, porosity and swelling ability. The good characteristic of scaffold could be obtain from synthesis and good fabrication technique.

1 Introduction

Tissue damage to the oral cavity can include tooth tissue damage, periodontal tissue damage and alveolar bone damage. Alveolar bone damage can be caused by trauma, tumors, congenital abnormalities, infection and bone retraction [1]. In addition, bone damage can also be found in several diseases, like rheumatoid arthritis, osteoporosis and osteolysis [2]. Bone has the ability to regenerate to form new bone if the defect is not a large defect. If the bone defect is large or it is also called a critical defect, the bone requires intervention and substitute materials to achieve optimal bone regeneration [3].

Tissue engineering technology or better known as tissue engineering is a multidisciplinary science that involves the principles and combinations of cell techniques, materials and biochemical and physicemical factors to restore, maintain and enhance biological functions [4]. This new innovation in tissue engineering techniques involves 3 components that have a regeneration success role, the 3 components are the scaffold (bone graft), molecular signals, and cells [5].

Scaffold is a material that is transplanted into damaged tissue with the aim of providing mechanical support as a frame for cells and signal molecules to work together to regenerate tissue by forming new tissue. The scaffold is designed to provide a microenvironment facility for cells so they can adhering, living, growing, proliferating and differentiating [6]. Based on the type of scaffolding they can be classified into autograft, allograft, xenograft and alloplastic graft (synthetic scaffold). Scaffold for bone regeneration is the second most common type of transplanted tissue, with more than 2.2 million transplants performed each year worldwide [7].

Synthetic scaffold material that can be used as an alternative is hydrogel gelatin. Gelatin hydrogel scaffold is a gelatin-based hydrogel which is made by crossing

the gelatin hydrogel by oxidation [8]. Gelatin is a natural product obtained from partial hydrolysis of collagen. Gelatin comes from cows (bones and skin), pork (skin only) and fish (skin), bovine gelatin was used in this study. Bone is a composite of collagen, a protein-based hydrogel template and an inorganic substance which is an osteoconductive component, therefore hydrogel polymers are the main choice to form functional scaffolds of tissue repairment[9].

The material used as a scaffold must be biocompatible, biodegradable, inhibits bone resorption and stimulates osteogenesis [10]. Hydrogel gelatin has been widely used in the health devices because it has good nature of biodegradable and biocompatible [11]. Hydrogel gelatin has many functions in tissue engineering. Hydrogel gelatin supports the angiogenesis process and provides proliferation and differentiation spot for cells [10]. In addition, the elastic hydrogel net provides unique mechanical properties including low stiffness, resistance of pulling and high resistance in fracture [12].

The process of cell attachment, proliferation and differentiation can run well if supported by the presence of growth factors that will regulate cellular occurrence in the wound healing process, in this case, bone damage. Growth factor can be obtained from platelet-rich plasma [13]. Platelet-rich plasma is a platelet with a high concentration with a limited plasma volume. Platelets contain seven growth factors, which is platelet derived growth factor (PDGF $\alpha\alpha$), PDGF $\beta\beta$, PDGF $\alpha\beta$, transforming growth factor beta (TGF- β), TGF- β 2, vascular endothelial growth factor (VEGF) and epithelial growth factor (EGF) [14]. The content of growth factor that has the most influence in bone regeneration is platelet derived growth factor (PDGF) which can increase endothelial cells that initiate capillary growth and transforming growth factor beta (TGF- β) which can increase osteoblasts and stem cells to initiate mitosis and osteoid production [15].

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PRP contact process with scaffold can occur at the time of loading the PRP. PRP incorporation process is the ability of scaffolding to be incorporated with PRP. Contact between the scaffold composer molecules with PRP and consequently the support of the scaffold degradation process causes growth factor release [16]. The loading process is very important as an initial stage prior to release and degradation process. The degradation process of the scaffold is influenced by the physicochemical properties of the scaffold designed to be biodegradable if not destroyed, the scaffold will be detected by the body as an isolate object so that the bone regeneration process is said to be unsuccessful [5].

PRP incorporation is a part of tissue engineering technology for bone tissue regeneration. The success of tissue engineering technology is influenced by a good scaffolding factor, the signal molecule in this case PRP which can unite or enter into the scaffold. An effective method of loading PRP against the scaffold is required. An effective PRP loading method must be able to load a large number of platelets [17]. The PRP loading method can be done by dip or drop. The dip method, also known as impregnated, has been carried out in several studies. Matsui and Tabata's study [5] used an dip method for loading PRP in hydrogel gelatin scaffolding and succeeded in increasing the number of blood vessels and the study of Nalawade et al. also used the dip method and was proven to be used in the rehabilitation of tooth displacement due permanent incisors injury [18].

The drop method has never been used before as a method of loading PRP on scaffold. This method may be more economical in using the signal molecules to be incorporated than the dip method. And a good method is one that can load a large number of platelets in a scaffold. If the platelet count is large, the more growth factors will be released and the regeneration process will run well [19]. This study will compare dip and drop methods for the incorporation of PRP into a hydrogel gelatin scaffold.

2 Material and Methods

Preparation of gelatin hydrogel scaffold

The scaffold is made from cow gelatin melted in aquadest. The solution obtained then printed to resemble a thin sheet. Cooled in the freezer, then continued by freeze drying. And to strengthen the structure, physical cross-linked with dehydrothermal was carried out.

Preparation of PRP

Platelet rich plasma was obtained from whole blood of 3 donors who had filled in the prior informed consent. Each donor had 10 ml of blood drawn and put in a vacutainer containing anti-coagulant citrate dextrose (ACD). PRP preparation was carried out by 2 times centrifugation at 4 ° C. First centrifugation with 450 rcf for 7 minutes, then the second centrifugation with 1600 rcf for 5 minutes. Each PRP of the 3 blood donors was incorporated into a different scaffold [5].

Incorporation PRP into scaffold

The PRPs of the 3 donors were coded differently and incorporated each PRP in 3 scaffolds. The dip method of incorporation was by preparing 50 ul of PRP on the microtube, then dipping the circle scaffold with a diameter of 6 mm into the base of the microtube and wait for 10 minutes. Meanwhile, the incorporated drip method, by dropping 50 ul of PRP using a yellow tip just above the scaffold and wait for 10 minutes. The scaffold that has been filled with PRP is removed, then the remaining PRP is counted by the number of platelets by using Giemsa's stain.

The method of calculating the number of platelets that are incorporated into the scaffold is to calculate the amount of initial PRP before incorporating, minus the remaining PRP in both methods, or by the following formula (Eq 1, Eq 2):

$$\text{The number of PRP loaded} = A - B \quad (1)$$

by dip method

$$\text{The number of PRP loaded} = A - C \quad (2)$$

by drip method

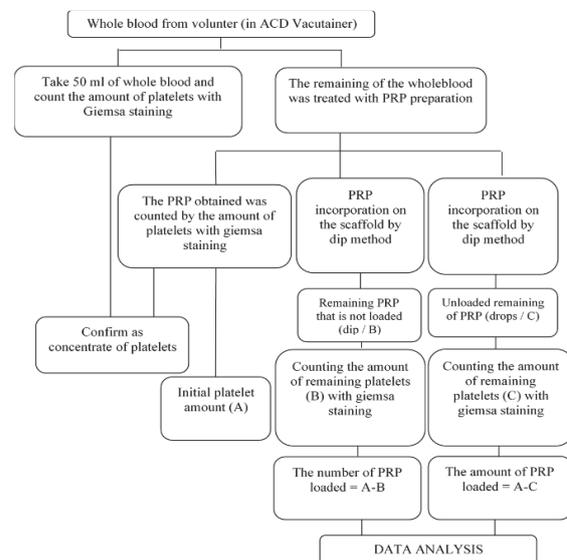
Information:

A: The number of platelets in the initial PRP before incorporated

B: The number of platelets after incorporating by the dip method

C: The number of platelets after incorporating by the drop method

The flow of this research is as follows:



3 Result and Discussion

Preparation of PRP from blood of 3 donors after counting the platelet by Giemsa's stain, obtained the results of each increase in the number of platelets in whole blood, as shown in Table 1.

Table 1. PRP Preparation platelete amount result

Blood Sample	Platelete amount(Thousand/mmk)
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	Whole Blood	PRP	PRP multiplying of Whole Blood
Donor 1	109	743	6,8165
Donor 2	178	564	3,1685
Donor 3	226	578	2,5575

This data represents the initial platelet amount before being incorporated and coded A. The difference between the number of platelets in whole blood (Figure 1a.) and the platelets in PRP after Giemsa staining (Figure 1b.) was seen using a light microscope at a magnification of 100 x. Platelets appear as small, nucleless, round cells with a pale purple to gray cytoplasm containing evenly distributed red-purple granules [20].

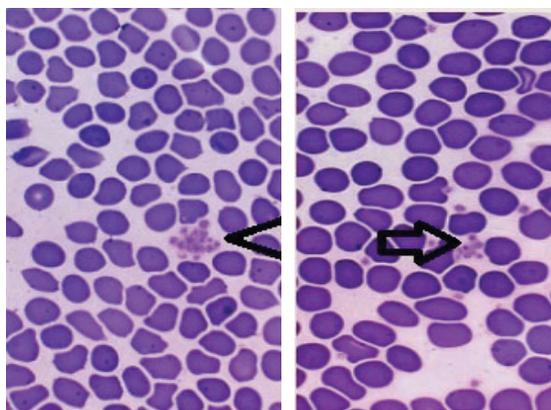


Figure 1a. Giemsa stain on whole blood

Figure 1b. Giemsa stain on platelet-rich plasma

Meanwhile, the incorporation results of PRP on the hydrogel gelatin scaffold by counting the amount of remaining platelets that were not incorporated into the scaffold are shown in Figure 2. The independent sample t test show the significant was 0.262 ($p > 0.05$), there's no significant difference between impregnated and drop method for incorporating PRP into gelatin hydrogel scaffold.

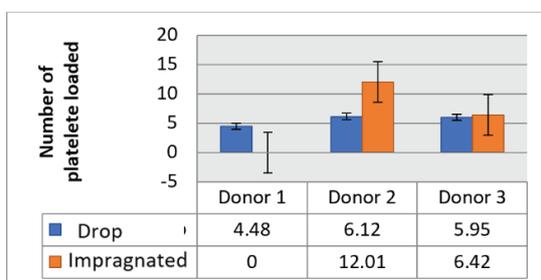


Figure 2. Incorporated PRP graphic on gelatin hydrogel scaffold

Gelatin hydrogel is a gelatin-based hydrogel that is made by hydrogel crossing. Hydrogel gelatin has been widely used in the health sector because of its good biocompatibility and biodegradability. Natural

polymers such as gelatin is known for better biomaterials for nerve regeneration, have adequate availability and have good physical-mechanical characteristics [21]. Moreover, the application of gelatin as a scaffold material in drug delivery systems is known to carry growth factors as bioactive compounds in bone regeneration [19].

There are many things that must be fulfilled in a scaffold, they are biocompatibility, biodegradability, mechanical properties, scaffold structure, interface adherence, porosity, processability, loading capacity release kinetic, stability and binding affinity. Regarding the ability to load platelet-rich plasma in hydrogel gelatin, the things that must be fulfilled are the structure of the scaffold, interface adherence and porosity. While the release process is greatly influenced by the loading capacity, kinetic release and binding affinity. Interface adherence is how cells able to attach to the surface of the scaffold and help cells in the process of adhesion and proliferation and facilitate the migration of cells. Interface adherence is influenced by the structure of the scaffold [22].

The structure of the scaffold illustrates that both macroscopically and microscopically the scaffold has a good surface and volume ratio that makes it easy for cells to adhere. The hydrogel surface morphology greatly influences the characteristics of the hydrogel membrane because the membrane surface directly interacts with fluids or the body's physiological environment [23]. The large proportion of gelatin can produce a hollow membrane surface. What determines the pore size is the volume of the polymer and the volume of solvent used. When the polymer combines with the solvent, the volume of the polymer floats while the volume of the solvent is fixed so that the difference will produce a porous pattern [24].

Swelling ability is related to the scaffold's ability in absorption process. Absorption is the ability of the scaffold to absorb fluids from the surrounding environment. The ability of the hydrogel to absorb water is influenced by the presence of free functional groups in the tissue of its molecular structure that can bind water. Several functional groups that have an effect on absorption are $-OH$, $-NH_2$, $-COOH$, $-CONH$ and $-SO_3H$ groups [25]. In addition, hydrogen bonds will be formed through the interaction between hydrogen atoms from water and oxygen atoms from the polymer. This is one of the factors that helps the water absorption mechanism in the hydrogel [26, 27]. The moisture content in the hydrogel is highly dependent on the concentration of the formula used in the scaffold fabrication [24]. The swelling mechanism of the hydrogel occurs because water diffuses by the osmotic pressure of the hydrogel. After reaching the equilibrium stage, the absorbed water will bond with the hydrogel groups to form hydrogen bonds. In the end, the absorbed water remains on the hydrogel so that the polymer undergoes swelling [28]. The water osmotic pressure is lower than the hydrogel osmotic pressure so that water will enter the hydrogel, because the substance will move from low osmotic pressure to high osmotic pressure [29]. As the characteristic of the gelatin, the hydrogel gelatin would swell after PRP was incorporated [30].

This is an important parameter to know because it has a correlation with the ability of the hydrogel to absorb platelet-rich plasma. The hydrogel membrane which expands in a liquid medium indicates that the polymer is able to absorb well [27,31].

The scaffold structure, porosity and interface adherence of each hydrogel gelatin scaffold will vary depend on the synthesis process and fabrication technique. Various types of fabrication techniques are used in scaffold design for tissue engineering applications such as the solvent casting method, gas foaming, particulate leaching and ice particle leaching which have advantages and disadvantages [26,31]. The ice particle leaching method produced hydrogel gelatin with good porosity, and this fabrication method was also used in this study. The ice particle leaching method by melting ice particles has the advantage that it can control the pore structure, can produce a thicker scaffold and can be applied to three-dimensional scaffold for bone regeneration in tissue engineering [26].

4 Conclusion

The impregnated and drop method is a method that can be used in the incorporation of PRP on the gelatin hydrogel scaffold. Both methods have no effect on the large number of platelets incorporated into the scaffold. The things that influence are the characteristics of the gelatin hydrogel scaffold itself are structure, porosity, interface adherence and swelling ability.

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