The Effect of Platelet-Rich Plasma (PRP)-Incorporated Synthetic Coral Scaffold to the Cessation of Bleeding

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Abstract. Bleeding is a normal body response after tooth extraction. Bleeding can be detrimental if occurs excessively. Excessive bleeding after dental treatment may result in shock, syncope, or even death if not treated properly. Hence, effective hemostatic agents are needed to prevent excessive bleeding. Various types of hemostatic agents have been developed by researchers and used by practitioners. The purpose of this study was to examine the effect of platelet-rich plasma (PRP)-incorporated synthetic coral scaffold to the time of bleeding cessation in Rattus norvegicus. This study employed posttest design to see the results of the treatment. There were four treatment groups: (a) Spongostan®, (b) PRP-incorporated synthetic coral scaffold, (c) synthetic coral scaffold, and (d) control (without hemostatic agent). Each group consisted of seven rats. Rat's tail was excised 2 cm from the tip, and then given treatment accordingly. Time of bleeding cessation was measured using a stopwatch, starting from the first drop of blood until blood was no longer spotted on filter paper. The results showed that PRP-incorporated synthetic coral scaffold group had the shortest cessation time (177.33 s), followed by Spongostan® (276 s) and only scaffold groups (314.67 s). Control group was the slowest (387.83 s). There was a significant difference between the control group and PRP-incorporated synthetic coral scaffold group ($p = 0.033$), but not significant with Spongostan® ($p = 0.403$) and only scaffold groups ($p = 0.726$). PRP-incorporated synthetic coral scaffold had the ability to reduce the time of bleeding cessation.

Keywords: Synthetic coral scaffold · Incorporation · Platelet-rich plasma · Time of bleeding cessation

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1 Introduction

Bleeding is one of the most frequent conditions following tooth extraction. It is the process of the blood flow out of blood vessels due to a disease or trauma. Post tooth extraction bleeding may causepanic to both doctors and patients [1]. Soft tissue bleeding is the second major tooth extraction complication (0–26%) after root fracture [2]. Studies reported that the most frequent complication after tooth extraction is excessive bleeding. Risk of bleeding complications is high on patients with liver disease, hypertension, platelet deficiency, hemophilia, factor VIII (von Willebrand factor) deficiency, or vitamin K deficiency [3, 4].

In dental practice, local hemostatic agents are required to accelerate the process of bleeding cessation after tooth extraction or other treatment that cause bleeding. Spongostan® is a commonly used local hemostatic agent in clinical practice. It is made from pure gelatin sponge or 100% porcine gelatin with the same density. Spongostan® can be absorbedby the body without causing any health issue. It is effective for controlling venous bleeding in the process of local hemostasis [5]. Nevertheless, it has several disadvantages in which it might cause hematoma, allergic reactions, extensive fibrosis, and toxic shock syndrome (TSS) [6].

In this modern age, technology in the world of medicine has seen advanced stage of development. One of the advanced technology is tissue engineering that enhances wound healing. Tissue engineering is an interdisciplinary field aimed at stimulating the growth of new tissue on damaged area. It is performed by combining the appropriate building blocks to initiate cells regeneration [7]. Tissue engineering refers to a discipline which studies the technology of developing artificial tissues or organs in order to restore, maintain, or improve the functions. In general, there are three factors that contribute to the success in tissue engineering technology: cells, scaffolds, and growth factors [8]. Scaffolds are one of the units in tissue engineering to support cells growth, and formulated by gelation technique on biohydrogel [9]. The prospective scaffolds require appropriate characteristics in accordance with the target tissue or organ, such as porosity, microstructure, macrostructure, biocompatibility, biodegradability, and mechanical properties which are compatible with the cells [8]. Furthermore, scaffolds also must have good absorption properties and structure that mimics the body's extracellular matrix (ECM) [10].

Calcium carbonate (CaCO3) is a compound that can be used as a scaffold in tissue engineering. Calcium carbonate has an excellent biocompatibility quality, and hence can be developed to be implemented in a bone graft. Combinations between gelatin in the form of hydrogel and calcium carbonate can create a scaffold with optimum biodegradability and biocompatibility properties [11]. Besides its role to provide microenvironment, calcium carbonate scaffold has the ability to release calcium ion which plays a role in hemostasis [12, 13].

The existence of platelets, or thrombocytes, produced in blood and bone marrow has a significant role in hemostasis, especially in blood clotting process when there is a wound. Initially, platelets showed the main function as the center in blood clotting process, but it was gradually apparent that they also have other important functions. They are involved as sources for various growth factors in the process of wound healing, acute response in tissue after trauma, and a few cellular physiological processes, such

as growth, differentiation, and cells replication [14]. Based on the important role of platelets especially in hemostasis, platelet-rich plasma (PRP) was developed. PRP is an autologous blood plasma in a small volume with highly concentrated platelets. Several growth factors which are actively secreted during wound healing process that can be found in PRP are: transforming growth factor beta (TGH-β), basic fibroblast growth factor (bFGF), platelet-derived growth factor-AB (PDGF-AB), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), and connective tissue growth factor (CTGF) [15].

A PRP-incorporated scaffold will have longer degradation time, and growth factor will perform on the target wound optimally as a consequence. On the basis of this, the development of a more effective local hemostatic agent to cease bleeding, and which does not cause health issue for patients (children), is necessary. This study aimed at examining the effect of scaffolds made from gelatin and calcium carbonate incorporated with PRP compared with commonly used local hemostatic agents in clinical practice, Spongostan®, on wound healing process of Rattus norvegicus based on time of bleeding cessation.

2 Materials and Methods

2.1 Subjects

The subjects of this study were 28 male healthy R. norvegicus (strain Wistar) aged 2–3 months and weighed 200–300 g. These rats were divided into four groups (7 rats in each group). They were acclimatized for 5 days before treatment. Standard feed and drink were given in individual cages. For the treatment, the rats were wounded until bleeding, and then the time of bleeding cessation on each treatment group were observed. The different treatment applied to the four groups was as follows: (a) Spongostan®, (b) PRP-incorporated synthetic coral scaffold, (c) synthetic coral scaffold, and (d) without hemostatic agent. The one way annova ($p < 0.05$) was used to anilize the data and followed by post hoc Tukey.

2.2 *Platelet-Rich Plasma and Scaffold Incorporation*

PRP was prepared by centrifugation in a refrigerated centrifuge (4 °C) twice: 450 RCF for 7 min, followed by 1,600 RCF for 5 min [16]. PRP rich in growth factors was ready to be incorporated into synthetic coral scaffold.

A synthetic coral scaffold is a scaffold developed from a mixture of calcium carbonate and bovine gelatin with aquadest (pure water from distillation process). Sodium citrate was used as dispersant, and the solution was solidified to form thin ¬film (Fig. 1a). It was then freeze dried and physical crosslinked using dehydrothermal (DHT) [17].

Synthetic coral scaffold was prepared in a dry state and sterile with length and width sizes of ± 7 mm. PRP of 40 ul was dropped on to the scaffold, then waited for 10 min to ensure full absorption of PRP into the pores of scaffold. Then, PRP-incorporated scaffold was ready to be applied at the wound site (Fig. 1b).

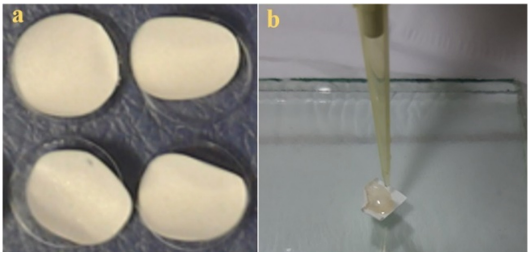

Fig. 1. Synthetic coral scaffold (a); PRP incorporation (b)

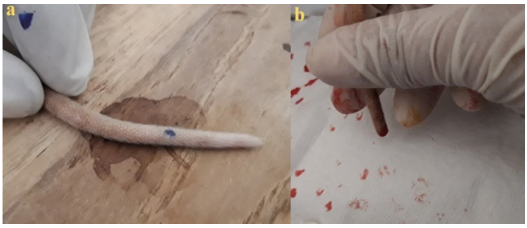

Fig. 2. Tail of rat to made the wound (a) and blood cessation measurement (b)

2.3 Wound Infliction and Bleeding

Wound was made on the tail of the rats at 2 cm from the tip measured by sliding caliper and marked with ink (Fig. 2a). Povidone-iodine on cotton buds was applied at the wound site to prevent infection. Topical anesthesia was administered using ethyl chloride spray with indication of crystal ice-like substance appearing on the tail. Excision on the marked tail was carried out using sterile scalpel.

2.4 *Treatment and Time of Bleeding Cessation Observation*

Wound care or treatment was conducted by applying hemostatic agents at the wound site. There were four different treatments: (a) Spongostan®, (b) PRP-incorporated synthetic coral scaffold, (c) synthetic coral scaffold, and (d) without hemostatic agent (only povidone-iodine). Hemostatic agent was directly applied at the wound site according to the different treatment, and then filter paper was placed at the wound for every 15 s until blood was no longer spotted on filter paper (Fig. 2b). Time of bleeding cessation was observed by measuring the time using a stopwatch starting from the first drop of blood after wound infliction until bleeding ceased.

3 Results and Discussion

Results showed different bleeding durations in the four different treatments. Different content in the applied hemostatic agents might affect the time until bleeding cessation. Figure 3 depicts the time of bleeding cessation for each group of treatment.

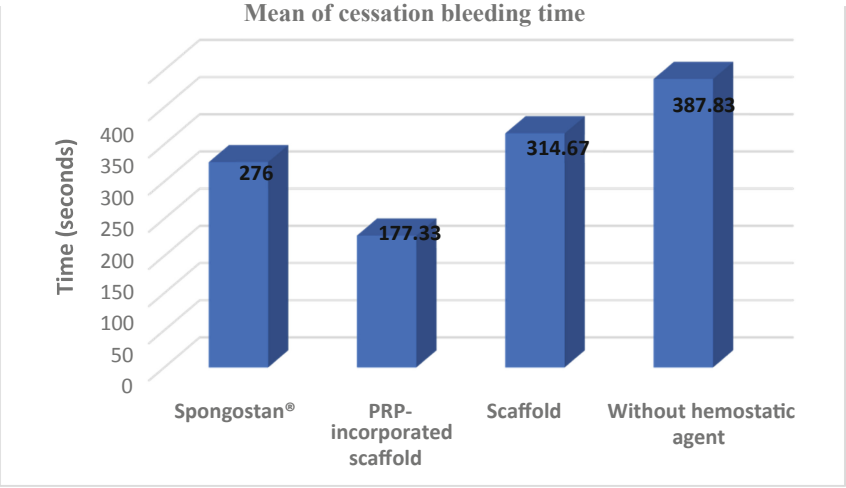

Fig. 3. Mean of time of bleeding cessation.

PRP-incorporated synthetic coral scaffold group showed the fastest bleeding cessation (177.33 s), followed by Spongostan® group (276 s) and then only scaffold group (314.67 s). Control group (without hemostatic agent) was the slowest (387.83 s). Statistical analysis showed significant differences between control group and PRP-incorporated synthetic coral scaffold group ($p = 0.033$), but not significant with Spongostan® ($p = 0.403$) and only scaffold groups ($p = 0.726$).

In this study, PRP-incorporated synthetic coral scaffold demonstrated to have the best hemostatic effect in terms of reducing the time of bleeding cessation. PRP has the ability to release growth factors, such as PDGF which is involved in vasoconstriction process. Vasoconstriction is a reflex response which occurs after blood vessels are damaged. This process is then maintained by local factors which are 5-hydroxytryptamine (5-HT), epinephrine, thromboxane A2 (TXA2), and PDGF [18]. Vasoconstriction is a hemostatic phase which is indicated by the constriction of damaged blood vessel walls, thereby reducing the volume of leaked blood [19]. In addition to the growth factors in PRP, synthetic coral scaffold also supports hemostasis due to its ability of releasing calcium ion [20] which is used as a co-factor in coagulation [13].

Physiologically, hemostasis is initiated by the adhesion of platelet to collagen fibers on subendothelium, and mediated by von Willebrand factor (vWF). vWF is a plasma protein which is naturally released by subendothelium and megakaryocytes soon after blood vessels ruptured. After platelets adhered to the subendothelial collagen fibers, platelets release adenosine diphosphate (ADP) as an initiator of primary platelet aggregation. This ADP has two roles: (1) facilitating adhesion of new platelets to form secondary platelet

aggregation and (2) attaching to platelets walls that have adhered to subendothelium and open fibrinogen receptor. Fibrinogen will bind to this receptor by calcium ion [21]. Fibrinogen attached to the subendothelium forms fibrin networks, and then cover the ruptured blood vessels, thereby ceasing bleeding [19]. Thus, calcium ion in hemostasis process functions as a catalyst in the formation of thrombin from prothrombin, mediating fibrinogen to bind to platelets adhered to the subendothelium, and assisting in the formation of fibrin threads with a stabilizer factor (factor XIII) [22].

Despite the fact that calcium ion is crucial in hemostasis process, results from this study did not show significant difference ($p = 0.726$) between the treatment of only using scaffold made from calcium carbonate and the control in terms of time of bleeding cessation. Bleeding cessation could be faster if there is other hemostatic agent, such as the use of PRP due to the existence of growth factors which are involved in vasoconstriction [18].

The second fastest group in ceasing bleeding was Spongostan®. This hemostatic agent is a gold standard which is commonly used for treating bleeding post tooth extraction. It is a popular hemostatic agent with easy application in dental practice [23]. Mechanism of action of this hemostatic agent is by absorbing blood 45 times its own weight, promoting platelet aggregation and wound closure, thereby ceasing bleeding [24].

The use of PRP, platelet poor plasma (PPP), and concentrated PPP to cease bleeding have also been demonstrated on pigs. PRP was shown to have significant hemostasis effect, followed by PPP and then concentrated PPP. This result was due to the higher platelet content in PRP [25]. Similar results were demonstrated in a study to cease bleeding topically on pigs' wound by using PRP, PPP, and without hemostatic agent. Using topical PRP, bleeding could cease faster [26]. Another similar study by Hasigawa who conducted PRP transfusion to examine the hemostatic effect on rabbits showed that PRP transfusion was effective in the cessation of bleeding due to the interactions between red blood cells and platelets [27]. The application of PRP at the wound site results in adequate hemostasis process with mean time of bleeding cessation approximately 3 min [23]. This time length is similar to the result of this study in which PRP-incorporated synthetic coral scaffold could stop bleeding in 2 min 57 s, thereby reducing time until cessation of bleeding as compared to other treatments.

4 Conclusion

PRP-incorporated synthetic coral scaffold showed the shortest bleeding cessation time compared to other treatments. This was due to the growth factors in PRP which are involved in vasoconstriction, and also the calcium ion in the scaffold which is crucial in coagulation process.

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