2018 1st International Conference on Bioinformatics, Biotechnology, and Biomedical Engineering (BioMIC), Yogyakarta, Indonesia

The Effects of Platelet Rich Plasma Incorporation Towards Swelling Profile and Gel Fraction of Synthetic Coral Scaffold

1st **Mahanani, Erlina Sih**
Biomedical Dental Science Dept., School of Dentistry, Faculty of Medicine and Health Science Universitas Muhammadiyah Yogyakarta
Yogyakarta, Indonesia
erlina.sih@umy.ac.id

2nd Farda, Nur
Dental Hospital, Faculty of Medicine and Health Science Universitas Muhammadiyah Yogyakarta
Yogyakarta, Indonesia
nurfarda@gmail.com

3rd Tejaningasih, Inten
Dental Hospital, Faculty of Medicine and Health Science Universitas Muhammadiyah Yogyakarta
Yogyakarta, Indonesia
intentejaningasih@gmail.com

4th Khairunissa, Nadya
Dental Hospital, Faculty of Medicine and Health Science Universitas Muhammadiyah Yogyakarta
Yogyakarta, Indonesia
nadyanissa94@gmail.com

***Abstract*— Platelet Rich Plasma (PRP) contains of bioactive molecule which is able to incorporate to scaffold for promoting bone healing. Scaffold will absorb PRP, with the result that it may affect the structural stability, shape, and degradation process itself. Consequently, this study aim is to observe the influence of incorporated PRP to synthetic coral scaffold towards the swelling profile and gel fraction. Platelet-rich plasma was prepared by a double-spinning method. The blood sample was taken from lateral tail vein of Rattus norvegicus. The synthetic coral scaffolds were made of gelatin and calcium carbonate (CaCO3). They were divided into two groups. First is PRP incorporation group and the second is non-PRP synthetic coral scaffolds as control group. The incorporation process was done by shedding scaffold into 70µl of PRP for 15 minutes. Swelling observation was examined by soaking scaffold in phosphate buffer saline and incubated in 37 C degrees for 24 hours. Scaffold weight was measured in every 30 minutes to observe the profile swelling and gel fraction. Used data analyzing was Independent T test. The result showed no significant differences between two groups. However, the initial measurement graphic showed the PRP incorporated scaffold swelling profile had higher number compared to the non-incorporated one. According to this study, it can be concluded that the incorporation of PRP in synthetic coral scaffold affects its swelling profile and gel fraction.**

Keywords*— *swelling, gel fraction, synthetic coral scaffold, platelet rich plasma, incorporation

I. INTRODUCTION

Bone damage which reaches a critical defect requires external intervention such as surgery and the application of tissue engineering technology for bone regeneration [1]. Tissue engineering has 3 factors that influence the success of tissue regeneration, i.e.: cell, scaffold, and molecule signal. Scaffold is essentially needed as cell's micro environment to proliferate, differentiate, until it generates the bone matrix [2]. Previous study has shown that natural sea coral is beneficial as a bone regeneration media. Besides, the exaggerating use of coral may threaten its habitat. Therefore, scaffold may be the wise alternative. It is an artificial coral-like framework that provides mechanical support for cells to perform their functions, forming new bone tissues. It was designated to mimic natural coral which has biodegradable and biocompatible properties. Those characteristics also grants scaffold to be loaded by molecule signal in inducting proliferation and differentiation process [3]. Platelet-rich plasma (PRP) is defined as a platelet concentration that contains bioactive molecules. It has been widely used in oral and maxillofacial surgery to promote bone healing. Growth factors can be obtained from PRP. Platelet concentration in plasma is rich of growth factors. Platelet-rich plasma can be used combined with scaffolds to aid bone regeneration [4]. However, when PRP is incorporated, it will cause scaffold to swell. Once it is implanted into body, the swelling process will continue due to body fluids. In the other hand, scaffold has an ability to absorb fluid and maintain structural stability of its shape until the gel fraction occurs so that scaffold degraded. Further information about swelling profile and gel fraction of synthetic coral scaffold is not available yet. Corresponding design is needed for bone regeneration [5]. Therefore, this study is needed to observe the effect of PRP incorporation on synthetic coral scaffold to swelling profile and gel fraction.

II. MATERIAL AND METHOD

Scaffold Preparation

Synthetic coral scaffold was made of gelatin (Nitta Co., Osaka, Japan) and calcium carbonate (CaCO3) from Wako (Osaka, Japan), in weight ratio 5 : 5. It is prepared as thick film-like scaffold with interconnected porosity in 1,5 cm diameter. Sodium citrate (Sigma-Aldrich, Germany) was used as dispersant in the solution. Scaffolds were frozen for 24 hours and freeze dried. The physical crosslinking was done to strengthen the structure of scaffold [6]. They were divided into two groups, PRP incorporation group and non-incorporation group. In advance of its using, scaffold's weight was stabilized using oven in 50ºC degrees. Initial weight of every scaffold was measured.

978-1-5386-7599-1/18/$31.00 ©2018 IEEE

PRP Preparation

Platelet-rich plasma is obtained from lateral vein tail of Rattus norvegicus blood (Fig.1a), wistar strain, that collected in the vacutainer tube contained acid citrate dextrose as anticoagulant (BD, USA). Isolation of PRP followed Matsui and Tabata method with twice centrifugation process, 450 rcf in 7 minutes, and 1600 rcf in 5 minutes at 4ºC degrees (Fig. 1b) [7].

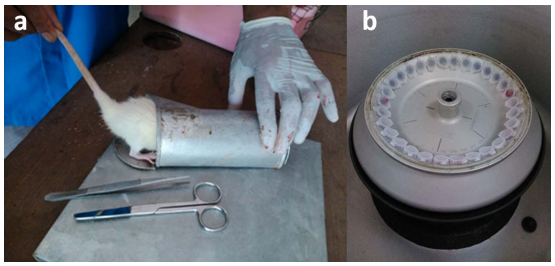

Fig. 1. Blood collection (a) and centrifugation (b) for PRP

Incorporation of PRP

The process of PRP incorporation on synthetic coral scaffold was done by drop method. The 70 µl of PRP was taken using a micropipette, dripped on scaffold . Wait for 15 minutes until PRP incorporated in the scaffold. Subsequently, the unabsorbed PRP residues was disposed using filter paper.

Measurement of SwellingCcapability

Scaffold was inserted in chamber containing phosphate buffer saline, and then being incubated in 37℃ degrees for 24 hours. Swelling profile was observed by measuring the scaffold's weight after absorbing liquids using laboratory balance for every 30 minutes, until the first 6 hours. Thereafter, it is being re-incubated until 24 hours to observe scaffold's gel fraction. Sample measurement was performed for three times. Swelling ratio can be calculated by the formula: Swelling ratio (%) = ((Ww-Wo) / Wo) × 100

Wo: initial weight , Ww: wet weight

Gel Fraction

The gel fraction was taken after 24 hours immersion, then samples were dried and weight-measured. The calculation of gel fraction percentage was measured by this formula below:

Gel fraction formulation (%) =Wd/Wi × 100
Wi: dry weight before immersion
Wd: dry weight after immersion
The data of gel fraction were analyzed by independent t test.

III. RESULT AND DISCUSSION

Result

Synthetic coral scaffold in Fig. 2 was fabricated as thick film-like with interconnected porosity, which makes platelet possibly incorporates in scaffold. Synthetic coral scaffold in Fig. 2 was fabricated as thick film-like, with interconnected porosity, make possible platelet to incorporate in scaffold.

Fig. 2. Thick film-like scaffold

The scaffold appeared to swell after being immersed in phosphate buffer saline. It was showed in Fig. 3.

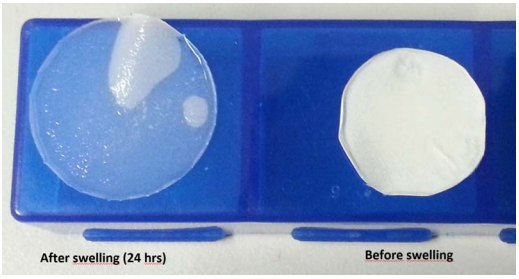

Fig. 3. Synthetic coral scaffold, before and after swelling

The swelling ability of incorporated and non-incorporated scaffold were obtained from swelling ratio formulation, it is shown in Table 1.

Table 1. Swelling ratio of synthetic coral scaffold

Time of immersion	∑ *Swelling ratio*	
	PRP	Non-PRP
30 min	597.76 %	380.85 %
1,0 hr	434.90 %	331.52 %
1.5 hr	390.79 %	262.39 %
2.0 hr	362.16 %	241.47 %
2.5 hr	334.28 %	233.58 %
3.0 hr	245.02 %	225.21 %
3.5 hr	228.64 %	218.55 %
4.0 hr	207.87 %	218.21 %
4.5 hr	197.83 %	218.21 %
5.0 hr	187.93 %	211.21 %
5.5 hr	187.13 %	206.46 %
6.0 hr	186.37 %	201.73 %
24 hr	185.70 %	197.98 %

The swelling profile between incorporated PRP and non-incorporated PRP could be observed in graph, which is presented in Fig. 4.

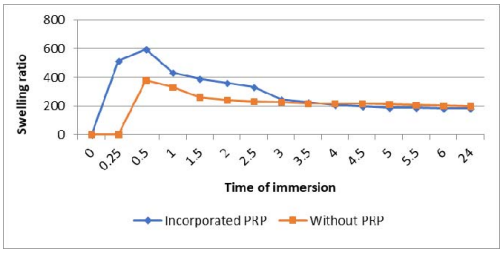

Fig. 4. Swelling profile

The graph shows the difference in swelling profiles between two groups. Scaffolds with incorporated PRP have higher swelling capability compared to non-PRP group until the 3rd hour. Then, both groups of scaffold samples tend to experience a gradual decrease in swelling capability. Besides

it tends to maintain its equilibrium, called the equilibrium phase until the 24th hour.

The data analysis of independent T test is shown on Table 2. Swelling ratio had significant differences over the 30-minute observation time and still had no significant difference until the end of observation. This due to scaffold in the equilibrium phase, so there is no increase of swelling.

Table 2. Independent t test of swelling rasio

Time of immersion	Sig. (2-tailed)
30 min	0.010*
1.0 hr	0.133
1.5 hr	0.103
2.0 hr	0.065
2.5 hr	0.105
3.0 hr	0.519
3.5 hr	0.692
4.0 hr	0.744
4.5 hr	0.363
5.0 hr	0.393
5.5 hr	0.449
6.0 hr	0.570
24 hr	0.668

The gel fraction of scaffold was observed after 24th hour, presented in Table 3. The results showed, that gel fraction of incorporated PRP group was higher than the non-PRP group. This is indicated by the weight of remaining scaffold which encounters gel fraction process.

Table 3. Gel fraction of scaffold

Samples	Gel Fraction (%)
Incorporated PRP 1	97.53
Incorporated PRP 2	94.81
Incorporated PRP 3	90.91
Without PRP 1	67.11
Without PRP 2	84.67
Without PRP 3	70.95

Discussion

The data analysis showed no significant differences between groups. However, the graph presented that there is a difference in swelling profiles movement between PRP and non PRP group. The PRP incorporated group was encountering a faster swelling process in the early of 3 hours compared without PRP. Generally, the swelling process of scaffolds increased in early time, then decreased due to the undergoing equilibrium until 24th hour, called equilibrium phase. It is supported by the previous study in PVA/HA hydrogel composite. The swelling process is divided into four stages, i.e.: increase rapidly, decrease, decrease slowly, and then stabilize [5]. The initial swelling is expected to increase porosity in facilitating cell attachment. However, continuous swelling causes loss of mechanical integrity and pressure to the surrounding area [8].

When synthetic coral scaffold incorporated to PRP, it will activate PRP, due to the presence of Ca ion. It releases growth factors that act as molecule signal [9]. Generally, the coagulation component in plasma will form a fibrin matrix with 3-dimensional formation that can act as a natural scaffold for cell attachment in tissue repair process [10]. Ca ion applies as a thrombin co-factor, it could activate the prothrombin into thrombin and change the fibrinogen (contained in PRP) into fibrin fibrils. It can be assumed that Ca ions in synthetic coral scaffolds will form fibrin fibrils as well. It is supported by Shimojo's study. Fibrin network could generate the electrostatic bonds, that strengthen the mechanical properties of scaffold [11].

PRP incorporated scaffold has stronger structures due to electrostatic bonding between fibrin network from PRP activation and ion Ca in scaffold [12]. Increasing mechanical strength of scaffold provides cell attachment, proliferation, differentiation for newly formed bone tissue [2].

After 24 hours of immersion in PBS, scaffold will undergo a structural breaking process called gel fraction. This process is characterized by the decreasing of scaffold mechanical properties which will soon be degraded. There is a difference in gel fraction percentage between synthetic coral scaffolds PRP incorporated and without PRP. Gel fraction value determines the final weight of scaffold in its dry state after immersion on PBS. In this case, the weightier dry scaffolds have higher gel fraction percentage [13, 14]. Results showed that gel fraction values on PRP incorporated scaffold had higher values compared to non-incorporated one. This means that PRP incorporated scaffold has stronger structures characterized by the weight of dissolving scaffold structure in the PBS less than non-PRP incorporated scaffold.

CONCLUSION

PRP incorporated synthetic coral scaffold has higher swelling profile and gel fraction percentage comparing the non-incorporated one, that demonstrated the stronger structures characteristic.

ACKNOWLEDGMENT

Authors thank to Universitas Muhammadiyah Yogyakarta by "Unggulan Prodi Research Funding 2017" that supported this study.

REFERENCES

[1] K. A. Blackwood, N. Bock, T. R. Dargaville, & M. A. Woodruff, "Scaffolds for Growth Factor Delivery as Applied to Bone Tissue Engineering," International Journal of Polymer Science, 2012, 1–25.

[2] F. J. O'Brien, "Biomaterials & scaffolds for tissue engineering," Materials Today, 14(3), 2011, 88–95.

[3] G. Chen, T. Ushida, and T. Tateishi, "Scaffold Design for Tissue Engineering," Macromol Biosci, 2002, 67-77.

[4] I. A. Rodriguez, E. A. Kalaf, G. L. Bowlin, and S. A. Sell, "Platelet-Rich Plasma in Bone Regeneration: Engineering the Delivery for Improved Clinical Efficacy," BioMed Research Internasional, 2014, 1-15.

[5] D. K. Zhang, D. G. Wang, J. J. Duan, and S.-r. Ge, "Research on the Long Time Swelling Properties of Poly (vinyl alcohol)/ Hydroxylapatite Composite Hydrogel," Journal of Bionic Engineering, 2009, 22-28.

[6] S. C. Leeuwenburgh, I. D. Ana, and J. A. Jansen,"Sodium Citrate as an effective dispersant for synthesis of inorganic-organic composites with nanodispersed mineral phase," Acta Biomater vol 6, No. 3, 2010, 836-844.

[7] M. Matsui, and Y. Tabata, "Enhanced angiogenesis by multiple release of platelet-rich plasma contents and basic fibroblast growth factor from gelatin hydrogels," Acta Biomaterialia, 8(5), 2012, 1792–1801.

[8] Y. Pan, S. Dong, Y. Hao, T. Chu, C. Li, and Z. Zhang, "Demineralized Bone matrix gelatin as scaffold for tissue engineering," Journal of Microbiology, 4(9), 2010, 865–70.

[9] D. J. Sánchez-González, E. Méndez-Bolaina, and N. I. Trejo-Bahena, Platelet-Rich Plasma Peptides: Key for Regeneration," International Journal of Peptides, 2012, 1-10.

[10] E. Anitua, M. Zaiduendo, M. Troya, S. Padilla, and G. Orive, "Leukocyte Inclusion within a Platelet Rich Plasma-Derived Fibrin Scaffold Stimulates a More Pro-Inflammatory Environment and Alters Fibrin Properties," PLOSONE 10(3), 2015, 1-15.

[11] M. Sadeghi-Ataabadi, Z. Mostafavi-pour, Z. Vojdani, M. Sani, M. Latifi, and T. Talaei-Khozani, "Fabrication and Characterization of Platelet-Rich Plasma Scaffolds for Tissue Engineering Applications," Materials Science & Engineering, 2016, 1-41.

[12] A. A. M. Shimojo, A. G. M. Perez, S. E. M. Galdames, I. C. D. S. Brissac, and M. H. A. Santana, "Performance of PRP associated with porous chitosan as a composite scaffold for regenerative medicine," Scientific World Journal, 2015, 1-13.

[13] N. Nagasawa, T. Yagi, T. Kume, and F. Yoshii, "Radiation Crosslinking of Carboxymethyl Starch," Carbohydrate Polymers, 58, 2004, 109–113.

[14] Y. Li, Y. Liu, S. Li, S., G. Liang, C. Jiang, and Q. Hu, "Novel Control of Gel Fraction and Enhancement of Bonding Strength for Constructing 3D Architecture of Tissue Engineering Scaffold with Alginate Tubular Fiber," Journal of Bioscience and Bioengineering Vol. 121, No. 1, 2016, 111-116.