Effect of rSLPI Amnion Membrane Application on Incision Wound of Rattus Norvegicus in Collagen and VEGF Expression

by Anita Yuliati

Submission date: 27-Jul-2020 12:15PM (UTC+0800)
Submission ID: 1362648837
File name: mnion_Membrane_Application_Elly_Munadziroh_Alexander_Nugraha.pdf (709.89K)
Word count: 2224
Character count: 12437

Effect of rSLPI Amnion Membrane Application on Incision Wound of Rattus Norvegicus in Collagen and VEGF Expression

Elly Munadziroh[1], R. Helal Soekartono[1], Rossa Bella Vennowusky Rafli[2], Anita Yuliati[1], Nadia Kartikasari[3]*

1. Department of Dental Material, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.
2. Undergraduate Student, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.
3. Departement of Prosthodontic, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.

Abstract

Amniotic membrane is one of the natural biomaterial that is widely used for wound healing which has Secretory Leukocyte Protease Inhibitor (SLPI) as an active component. One of rSLPI functions is suppressing several pro-inflammatory factors, such as serine protease. Supression of serine proteinase in wound healing process may increase the amount of vascular endothelial growth factor (VEGF) and collagen.

The aim of this study is to evaluate the effect of rSLP amniotic membrane fluid application on incision wound of Rattus norvegicus in expression of VEGF and collagen.

The rats divided into 4 groups: control group and treated group with rSLPI (P1 0.03 cc, P2 0,045 cc, and P3 0.06 cc) (n=6). Insicion was made on rat's cervical back. On the day 4, tissues around insicion wound are collected for Masson's Trychrome (MT) and Immunohistochemistry (IHC) analysis. Expression of VEGF and collagen are calculated using microscope with 400x magnification.

Kruskal-Wallis test of VEGF showed significant value with p=0,006 (p<0.05). Mann-Whitney test of VEGF showed a significant difference between control and treatment group. Oneway ANOVA test result for collagen has p value 0.000 (p<0.05). Honestly Significant Difference (HSD) test showed a significant differences between all group.

Application of rSLPI amniotic membran fluid in Rattus norvegicus incision wound may increase VEGF and collagen.

Experimental article (J Int Dent Med Res 2017; 10(2): pp. 239-243)

Keywords: Amniotic membrane, rSLPI, VEGF, collagen.

Received date: 19 April 2017 **Accept date:** 13 May 2017

Introduction

The incision is one of basic procedure that used in oral treatment. Almost all of oral surgery procedure involves incision caused tissue damage. The body will recover through the wound healing process. Wound healing is a complex and dynamic process. Wound healing process is divided into three basic phases, hemostasis and inflammation, proliferation, maturation and remodeling[1].

Corresponding author:
Elly Munadziroh, DDS, M.Sc., Ph.D,
Departement of Dental Material, Faculty of Dental Medicine, Universitas Airlangga, Jl. Mayjen Prof. Dr. Moestopo 47, Surabaya, Indonesia 60132
E-mail: elly-m@fkg.unair.ac.id

The proliferative phase may induce vascular network restoration and granulation tissue formation[2]. In this phase, vascular network restoration is important since nutrients and oxygen are needed during wound repair. Blood vessel formation (angiogenesis) is initiated by growth factors, vascular endothelial growth factor (VEGF)[3]. VEGF is a potent proangiogenic factor that has been shown to contribute 50% or more of the proangiogenic activity in wounds[4]. VEGF was increased within 24 hours until 7th day after the injury and then decreased significantly[5]. Proliferative phase starts from 4 - 20 days after the injury. In this phase, fibroblasts may form collagen and connective tissue. Collagen was detected on the day 3 after injury[6,7,8].

Secretory Leukocyte Protease Inhibitor (SLPI) is member of acidic protein family. SLPI is an important key in epithelial tissue protection

from serine protease such as trypsin, leukocyte elastase, and cathepsin G[2,9]. SLPI has a function to accelerate wound healing[2,10]. Previous study showed application of SLPI in incision wound on 0.03 cc, 0.45 cc and 0.06 cc concentration may increase the number of macrophages and fibroblasts[6]. SLPI is finding in various body secretions such as seminal plasma, cervical mucus, breast milk, tears, saliva, and bronchial secretions[2]. Previous study showed amnion membrane had SLPI as active component[10].

Physiologically, concentration of SLPI is very low, recombinant expression is required to produce sufficient amounts for detailed biochemical characterization and biomedical applications[2]. Escherichia coli has previously been utilized as a host for heterologous production of recombinant SLPI (rSLPI)[2,10]. The aim of this study is to analyze the effect of rSLP amniotic membrane fluid application on incision wound of Rattus norvegicus in expression of VEGF and collagen on the day 4.

Materials and methods

Experimental Design

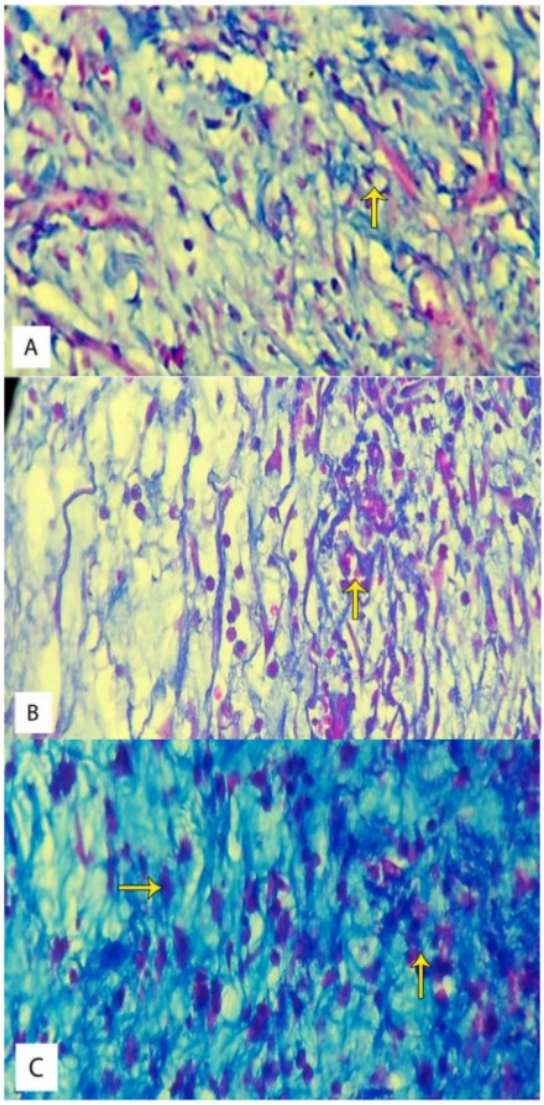
This study was an experimental laboratory using post test only control group design. This study was conducted in experimental animals using Rattus norvegicus as sample. This research had been approved with ethical clearance from Committee of Ethical Clearance of Health Research, Faculty of Dental Medicine, Universitas Airlangga, number 270/ KKEPK.FKG/IX/2015. Criteria for samples are male Rattus norvegicus 2-3 months with 250-300 gram weight. rSLPI obtained from Proteomic Laboratory from Institute of Tropical Disease, Universitas Airlangga.

Animal Treatment

Before the experiment begins, the rat was given adaptation in a cage (60 cm x 65 cm x 80 cm) for a week. The rats divided into 4 groups: control group and treated group with SLPI (P1 0.03 cc, P2 0,045 cc, and P3 0.06 cc). Each group consists of 6 rats. Before incision wound made, rat's hair has been removed. Insicion was made on rat's cervical back with leght 1 cm and deep 1 cm under the effect of anaesthesia intramuscular of ketamine (Kepro, Daventer, Holland) with dose 3-5 mg/kg BB. Histopatologic analysis (HPA) is conducted using immunohistochemia (IHC) staining for VEGF (Bioss, Massachusetts, USA) and Masson's Trychrome (MT) staining for collagen. HPA preparation was observed with 400x magnification to measure the expression of VEGF and collagen. The obtained data was analyzed by Kruskal-Wallis test for VEGF and Oneway ANOVA for collagen.

Results

Expression of VEGF could be seen in Figure 1 while collagen expression could be seen in Figure 2.



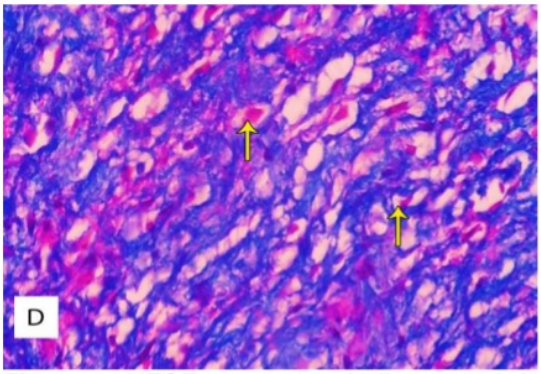


Figure 1. VEGF expression (yellow arrow) in light microscope 400x magnification in control (a), P1 (b), P2 (c), and P3 (d) group.

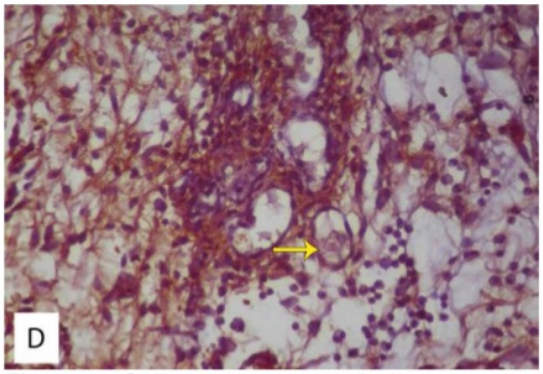
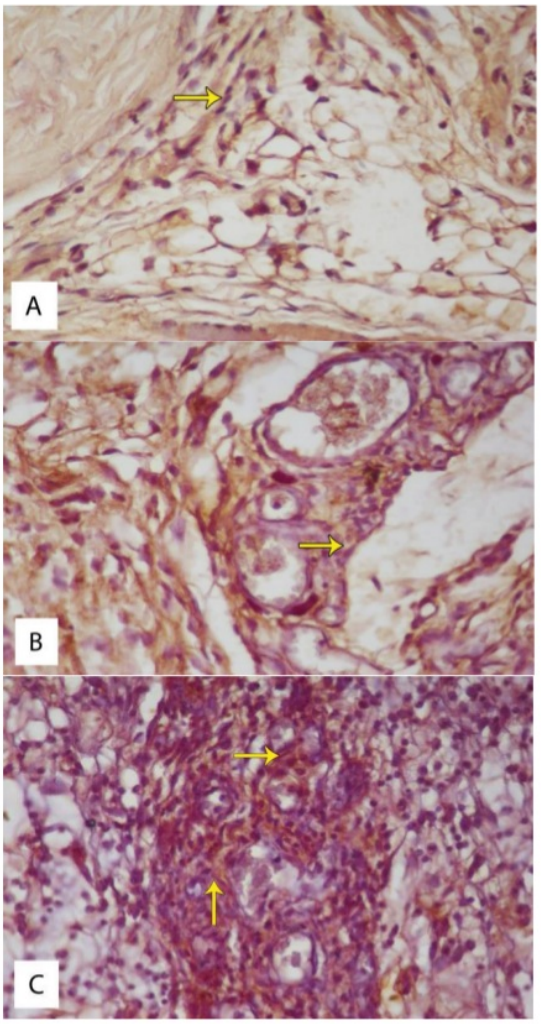


Figure 2. Collagen expression (yellow arrow) in light microscope 400x magnification in control (a), P1 (b), P2 (c), and P3 (d) group.



The average and standard deviations (SD) between the amount of VEGF and collagen from control group and the treatment group in wounded can be seen in the Table 1.

Group	n	VEGF		Collagen	
		Mean	SD	Mean	SD
Control	6	11,83	1,169	0,83	0,408
P1	6	13,67	1,966	0,00	0,000
P2	6	20,17	5,742	2,17	0,408
P3	6	21,67	3,933	3,00	0,632

Table 1. Mean and standard deviation of VEGF dan collagen.

Data of VEGF had normal distribution and heterogen. Due to this result, Kruskal-Walli's test should be conducted. The result of Kruskal-Walli's test showed that p value is 0.006 with significance level α = 5%. Differences between treatment groups was analyzed by Mann-Whitney shows in Table 2.

Group	Contro l	P1	P2	P3
Control	-	0,059	0,015(*)	0,006(*)
P1	-	-	0,076	0,036(*)
P2	-	-	-	0,422
P3	-	-	-	-

(*) = Significant level (p<0,05)

Table 2. Results of Mann-Whitney Test of VEGF.

Oneway ANOVA test was performed for the data of collagen. The results showed p value

is 0.000 (p<0.05). Differences between groups of collagen was analyzed using Tukey HSD (Honestly Significant Difference). The result of HSD test can be seen in Table 3.

Group	Control	P1	P2	P3
Control	0,015(*)	0,015(*)	0,00(*)	0,00(*)
P1	-	-	0,00(*)	0,00(*)
P2	-	-	-	0,015(*)
P3	-	-	-	-

(*) = Significant level (p<0,05)
Table 3. Tukey HSD Test of Collagen.

Discussion

SLPI is a multifunctional protein in the host defense response. SLPI is an importance factor which regulates the innate an adaptive immunity. SLPI may protect our body through several mechanism (i) as an antimicrobial agent, (ii) as an controller of inflammatory mediators and protects the host from excessive tissue damage by proteolytic enzymes released during inflammation, (iii) as suppressesor of inflammatory responses, (iv) regulates the production and pro-immunogenic function of neutrophil extracellular traps, and (v) it fosters repair and is a component of the molecular machinery that controls cell growth, differentiation and apoptosis. The main fuction result of SLPI actions in wound healing process is to counteract excessive inflammatory responses and to initiate healing processes[9,10].

Wound healing process is a complex process which devided into several phase (inflammation, proliferation, and remodeling)[11,12]. SLPI may also play an important role in all phase of wound healing process. Previous study showed that SLPI-null mice has prolonged plasma clotting time[13]. Another research also shown that cutaneous wound without exogenous application of SLPI may delay in skin wound healing[12,13].

The result of this study showed that VEGF expression is increased with the increasing concentration of rSLPI. VEGF is produced by macrophage and play an important role for angiogenesis[10]. This might be happened due to application of rSLPI might decrease pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), and tumor necrosis factor beta (TNF-β), Previous research showed that the absence of SLPI may increase elastase activity, excessive and prolonged inflammation, enhanced activity (but not expression) of TNF-β, and elevated expression of TNF-α[12,13,14].

In this study, collagen levels increased along with increasing concentration of rSLPI. Increased collagen expression might happen due to SLPI be able to inhibit elastase. Elastase may decrease variety of proteins such as collagen and fibronectin. In Addition, elastase also decreases Growth Factor (GF) and cytokine. Fibroblasts migrate to the injured area then increase of collagen amount. Fibroblasts produce extracellular matrix and replaced by collagen. Collagen will form a new network and wound healing process start[10,14,15].

SLPI stimulate the formation of Hepatocyte Growth Factor (HGF). HGF is a cytokine that produced by mesenchymal cells. This cells play an important role for the regulation of mitogenesis, motogenesis and morphogenesis. In addition, SLPI also affect fibroblast ability to contract collagen gels. Contraction of collagen gel was the result of fibroblast to organize and solidify the collagen fibers[10,13,16].

Conclusions

Application of rSLPI amniotic membran fluid in Rattus norvegicus incision wound may increase VEGF and collagen in wound healing process.

Declaration of Interest

The authors report no conflict of interest and the article is not funded or supported by any research grant.

References

1. Velnar T, Bailey T, Smrkolj V. The Wound Healing Process: an Overview of The Cellular And Molecular Mechanisms. J Int Med Res. 2009; 37: 1528–42.
2. Li Z, Moy A, Sohal K, et al. Expression and Characterization of Recombinant Human Secretory Leukocyte Protease Inhibitor (SLPI) Protein from Pichia Pastoris. Protein Expr Purif. 2009; 67:175–181.
3. Landén NX, Li D, Ståhle M. Transition from Inflammation to Proliferation: a Critical Step During Wound Healing. Cellular and Molecular Life Sciences. 2016;73: 3861–3885.
4. Koh TJ, DiPietro LA. Inflammation and Wound Healing: The Role of The Macrophage. Expert Rev Mol Med. 2013; 13:1-14.
5. Thorne CHM, Gurtner GC, Chung K, Gosain A, Mehrara B, Rubin P, Spear SL. Grabb and Smith's Plastic Surgery. 7th ed. Philadelphia: Wolters Kluwer. 2013:15-22.

6. James C, Cecil GAF. Oral Health Resources & Information for Kansas medical professionals. Kentucky Departement for Public Health and The University of Kentucky College of Dentistry. 2006: 23-45.
7. Larjava H. Oral Wound Healing–Cell Biology and Clinical Management.1st ed. Wiley-Blackwell. 2012: 2-8, 12-14, 40-43.
8. Moraes L.T., Trevilatto P.C., Grégio A.M.T., Machado A.M.N., de Lima A.A.S., Quantitative Analysis of Mature and Immature Collagens during Oral Wound Healing in Rats Treated by Brazilian Propolis. Journal of International Dental and Medical Research. 2011;4(3):106-110.
9. Majchrzak-Gorecka M, Majewski P, Grygier B, Murzyn K, Cichy J. Secretory Leukocyte Protease Inhibitor (SLPI), a Multifunctional Protein in The Host Defense Response. Cytokine and Growth Factor Reviews. 2016;28: 79–93.
10. Munadziroh E. Characteristic, ekspression, and Gen cloning code protein secretory leucocyte protease inhibitor membrane amnion as candidate for wound healing process in gingiva. Dissertation. Faculty of Dental Medicine : Universitas Airlangga. 2008: 23-45.
11. Gonzalez ACO, Costa TF, Andrade ZA, Medrado ARAP. Wound healing - A literature review. An Bras Dermatol. 2016:91, 614–620.
12. Indrani D.J., Lukitowati F., Darwis D., Effect of Gamma-ray Irradiation on Bacterial Penetration Power of Chitosan/Collagen Blend Membranes for Wound Dressing. Journal of International Dental and Medical Research. 2016;9(3):202-206.
13. Schulze H, Korpal M, Bergmeier W, Italiano JEJr, Wahl SM, Shivdasani RA.Interactions between the megakaryocyte / platelet-specific beta1 tubulin and the secretory leukocyte protease inhibitor SLPI suggest a role for regulated proteolysis in platelet functions. Blood. 2004; 104(13):3949-57.
14. Ashcroft GS, Jeong MJ, Ashworth JJ, Tumor necrosis factor-alpha (TNF-α) is a therapeutic target for impaired cutaneous wound healing. Wound Repair Regen. 2012; 20: 38–49.
15. Rosdiani A.F., Widiyanti P., Rudyarjo D.I. Synthesis and Characterization Biocomposite Collagen-Chitosan- Glycerol as Scaffold for Gingival Recession Therapy. Journal of International Dental and Medical Research. 2017; 10 (1):118-122.
16. De-Mendonça RJ, Coutinho-Netto J. Aspectos celulares da cicatrização. Anais Brasileiros de Dermatologia. 2009;84: 257–262.

Effect of rSLPI Amnion Membrane Application on Incision Wound of Rattus Norvegicus in Collagen and VEGF Expression

ORIGINALITY REPORT

25%	14%	17%	0%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Monika Majchrzak-Gorecka, Pawel Majewski, Beata Grygier, Krzysztof Murzyn, Joanna Cichy. "Secretory leukocyte protease inhibitor (SLPI), a multifunctional protein in the host defense response", Cytokine & Growth Factor Reviews, 2016 Publication	4%
2	Zhiguo Li, Allison Moy, Kirti Sohal, Carolyn Dam et al. "Expression and characterization of recombinant human secretory leukocyte protease inhibitor (SLPI) protein from Pichia pastoris", Protein Expression and Purification, 2009 Publication	3%
3	worldwidescience.org Internet Source	2%
4	ppjpi.unair.ac.id Internet Source	2%
5	ejfs.springeropen.com	

	Internet Source	1%
6	Taufan Bramantoro, Nafilah Karimah, Agung Sosiawan, R Darmawan Setijanto et al. "Miswak users' behavior model based on the theory of planned behavior in the country with the largest Muslim population", Clinical, Cosmetic and Investigational Dentistry, 2018 Publication	1%
7	Scott Maxson, Erasmo A. Lopez, Dana Yoo, Alla Danilkovitch-Miagkova, Michelle A. LeRoux. "Concise Review: Role of Mesenchymal Stem Cells in Wound Repair", STEM CELLS Translational Medicine, 2012 Publication	1%
8	www.scielo.br Internet Source	1%
9	exocorriges.com Internet Source	1%
10	utmb.influuent.utsystem.edu Internet Source	1%
11	Farahnaz Fahimipour, Mina Mahdian, Behzad Houshmand, Mohammad Asnaashari et al. "The effect of He-Ne and Ga-Al-As laser light on the healing of hard palate mucosa of mice", Lasers in Medical Science, 2012	1%

	Publication	
12	www.scientiasocialis.lt Internet Source	1%
13	ejournal.poltekkes-smg.ac.id Internet Source	1%
14	Retno Indrawati Roestamadji, Nahdah Izdihar Nastiti, Meircurius Dwi Condro Surboyo, Anis Irmawati. "The Risk of Night Shift Workers to the Glucose Blood Levels, Saliva, and Dental Caries", European Journal of Dentistry, 2019 Publication	1%
15	thieme-connect.com Internet Source	1%
16	"Platelets and Megakaryocytes", Springer Science and Business Media LLC, 2012 Publication	<1%
17	www.frontiersin.org Internet Source	<1%
18	www.jove.com Internet Source	<1%
19	spesialis1.ibs.fk.unair.ac.id Internet Source	<1%
20	e-journal.unmas.ac.id Internet Source	<1%

21	A. A. Kompiang Martini, Agung Nova Mahendra. "Administration of 50% propolis ethanolic extract increases the number of gingivitis fibroblast in H2O2-induced rats", AIP Publishing, 2019 Publication	<1%
22	Xuemin Li, Jianhua Wang, Guanghao Su, Zhimin Zhou, Jiawei Shi, Lingrong Liu, Man Guan, Qiqing Zhang. "Spatiotemporal control over growth factor delivery from collagen-based membrane", Journal of Biomedical Materials Research Part A, 2012 Publication	<1%
23	www.labome.org Internet Source	<1%
24	Putri Nurfriana, Prihartini Widiyanti, Djoni Izak Rudyardjo. "Effect of Collagen-Chitosan-Glycerol Composition in Scaffold for Gingival Recession Therapy", Journal of Biomimetics, Biomaterials and Biomedical Engineering, 2019 Publication	<1%
25	"Cellular and Molecular Control of Neuronal Migration", Springer Science and Business Media LLC, 2014 Publication	<1%

Exclude quotes	Off	Exclude matches	< 7 words
Exclude bibliography	On		

Effect of rSLPI Amnion Membrane Application on Incision Wound of Rattus Norvegicus in Collagen and VEGF Expression

GRADEMARK REPORT

FINAL GRADE

/0

GENERAL COMMENTS

Instructor

PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5