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Hyaluronic Acid Accelerates VEGF and PDGF Release from Advance Platelet Rich Fibrin in Diabetic Foot Ulcer

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ABSTRACT

BACKGROUND: Hyaluronic acid (HA) is an essential component of extracellular matrix and mediates signaling in wound healing. HA could induce growth factor release from Advanced Platelet Rich Fibrin (A-PRF), including Vascular Endothelial Growth Factor (VEGF) and Platelet-derived Growth Factor (PDGF). However, concentrations of the released-VEGF and PDGF have not been clearly disclosed. Therefore, current study was conducted to measure the release of these growth factors in HA + A-PRF gel of diabetic foot ulcer (DFU) subjects.

METHODS: Twenty DFU subjects were included in the study and treated with A-PRF or HA+A-PRF. A-PRF was derived from autologous peripheral blood and processed with low-speed centrifugation. HA was added with a ratio of 1:0.6. A-PRF or HA + A-PRF was applied topically on DFU. Upper tips of A-PRF or HA + A-PRF gels were collected on day 0, 3 and 7 for measurements of VEGF and PDGF concentrations with Enzyme-linked Immune-sorbent Assay (ELISA) methods.

RESULT: On day-3, both VEGF and PDGF concentrations of HA + A-PRF group were significantly higher than the VEGF (p=0.000) and PDGF (p=0.019) concentrations of A-PRF group. The VEGF and PDGF concentrations were continuously and significantly increased on day-7 of HA + A-PRF group, compared to the VEGF (p=0.000) and PDGF (p=0.004) concentrations of A-PRF group.

CONCLUSION: Combination HA+A-PRF induces VEGF and PDGF release from A-PRF. A mixture of A-PRF and HA could be more effective than A-PRF alone for treatment of DFU.

Keywords: hyaluronic acid, advanced platelet rich fibrin, PRF, growth factor, VEGF, PDGF, diabetic foot ulcer

Introduction

In tissue regeneration, the damaged tissues could be recovered by using a stable fibrin (1), mesenchymal cells, fibroblasts, and epithelial cells.(2,3) Autologous platelet with high concentrations of fibrin is widely used in tissue regeneration (4-6) as adjuvant in oral and maxillofacial surgery, sports medicine, orthopedic surgery, and aesthetic plastic surgery in the last decade (7-9). To promote tissue healing and regeneration, platelet concentrates are also used to obtain local release of growth factors.(10) In addition, platelet concentrates have been shown to enhance and stimulate the wound healing process and accelerate angiogenesis.(11)

Platelet concentrates are classified as platelet rich plasma (PRP) or platelet rich fibrin (PRF). Several studies have reported the suboptimal benefit of autologous platelet gel in the healing of diabetic foot ulcer (DFU). This is due to the low level of growth factor contained in the platelet gel, hyperglycemia, chronic inflammation, and high protease enzymes on the surface of DFU.(12) Therefore, better PRF quality should be produced. With low-speed centrifugation, the red blood cell, and its components such as fibrin, platelets, growth factors, leukocytes, and other circulating cytokines and proteins, can be separated and concentrated, so that advanced PRF (A-PRF) could be obtained.(13,14)

Hyaluronic acid (HA) is an essential component of extracellular matrix (ECM), therefore the addition of HA in A-PRF could improve wound healing-related ECM signaling.(15) Hyaluronic acid was reported to induce growth factors release for angiogenesis such as Vascular Endothelial Growth Factor (VEGF) and Platelet-derived Growth Factor (PDGF) (16), which will be crucial in DFU healing. However, concentrations of the released-VEGF and PDGF have not been clearly disclosed. Therefore, current study was conducted to measure the release of these growth factors in HA and A-PRF gel of DFU subjects.

METHODS

Study Design

An open label randomized controlled study had been conducted from July 2019 until April 2020 in Koja District Hospital, Jakarta and Gatot Soebroto Army Hospital, Jakarta. Informed consent was obtained from all subjects. DFU subjects with ulcer area <40 cm^2, categorized as Wagner 2, were included. Other inclusion criteria were blood sugar under control within the range of 150-200 mg/dL, taking oral antidiabetic medication, and hemoglobin A1c (HbA1c) within 6-7.5 mg/dL. Meanwhile, subjects with platelet dysfunction syndrome, thrombocytopenia, *thrombotic thrombocytopenic purpura* (TTP), unstable hemodynamic or pregnancy, were excluded.

Twenty mL of peripheral blood was withdrawn on selected subjects, followed by A-PRF production. A-PRF with/without HA were used to treat DFU topically. The study protocol was approved by The Ethics Committee of the Faculty of Medicine Universitas Indonesia (No. 0855/UN2.F1/ETIK/2018).

The A-PRF and HA+ A-PRF Fibrin Gels Production

Blood was centrifuged at 200xg for 8 minutes. Resulted erythrocytes layer was carefully removed. A-PRF was gradually formed by fibrin and buffy coat. Fibrin clot formed in center of the tube was collected and used for the treatment. For making the mixture of A-PRF and HA, 1 mL of A-PRF was added with 0.6 mL of 0.2% HA in vaseline and vortexed. The mixture was placed in a clean cup and ready to be used for the treatment.

Measurement of VEGF and PDGF Concentrations

To measure the concentration of VEGF and PDGF, on each day 0, 3 and 7, small samples were collected in transfer medium containing 0.9% NaCl by cutting the upper tips of A-PRF and

HA+A-PRF fibrin gels. The samples were centrifuged, lysed, and kept in -70ºC storage. For VEGF measurement, Human VEGF Enzyme-linked Immune-sorbent Assay (ELISA) Kit (Catalog No.: MBS355343, MyBiosource, San Diego, CA, USA) was used. Meanwhile for PDGF measurement, Human PDGF-AA ELISA Kit (Catalog No.: MBS2506128, MyBiosource) was used. Briefly, both kits were based on sandwich ELISA technology with detection range of 31.2-2000 pg/mL (for VEGF) and 15.63-1000 pg/mL (for PDGF), sensitivity: <1 pg/mL (for VEGF) and 9.38 pg/mL (for PDGF). Anti-VEGF or anti-PDGF-AA polyclonal antibody was pre-coated onto 96-well plates and the biotin conjugated anti-VEGF or anti-PDGF-AA polyclonal antibody was used as detection antibodies. Avidin-Biotin-Peroxidase Complex was added. The 3,3',5,5'-Tetramethylbenzidine (TMB) substrate was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The optical density (OD) of yellow was measured at 450nm in a microplate reader, and then the concentration of VEGF and PDGF were calculated.

Statistical Analysis

All calculated datas of VEGF and PDGF were presented as mean±SD. Statistical analyses were performed using SPSS for Windows software version 20 (IBM, Armonk, NY, USA). The data were analysed using SPSS version 20. The p value <0.05 was considered as significant.

RESULTS

Twenty subjects with DFU were selected and randomly divided into two groups for A-PRF or HA + A-PRF fibrin gel application. A-PRF fibrin gel-treated subjects were 6 women and 4 men with

118 average age of 64, while HA + A-PRF-treated subjects were 5 women and 5 men with average age
119 of 59.

120 ***VEGF Concentration in Fibrin Gel of A-PRF and HA + A-PRF Groups***

121 As shown in Figure 1, on day-0, the VEGF concentration of A-PRF group (137.11±119.45 pg/mL)
122 was lower than the VEGF concentration of HA + A-PRF group (181.75±160.87 pg/mL), but not
123 significant (p=0.226, Mann-Whitney test). On day-3, VEGF concentration of HA + A-PRF group
124 (910.62±307.35 pg/mL) was significantly higher (*p*=0.000, Mann-Whitney test) than the one of
125 A-PRF group (279.99±141.49 pg/mL). The VEGF concentration was continuously and
126 significantly increased on day-7 (*p*=0.000, Mann-Whitney test), the VEGF concentration of HA +
127 A-PRF group (1,105.60±344.39 pg/mL) compared to the one of A-PRF group (436.16±269.93
128 pg/mL). The VEGF concentrations were further analyzed by measuring the increment (Δ) of
129 VEGF concentration on day-3 and day-7 with subtraction of baseline (day-0) (Table 1). The
130 ΔVEGF concentration of HA + A-PRF group was significantly higher than the one of A-PRF
131 group for both Δ0-3 and Δ0-7.

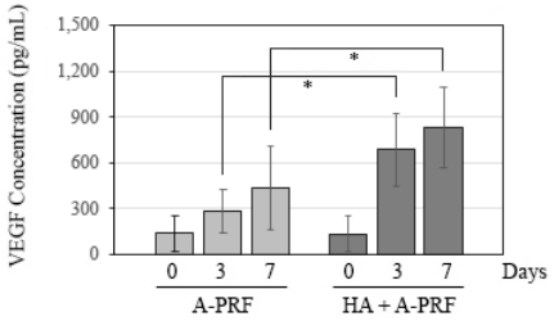

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Figure 1. VEGF concentrations of Fibrin Gels. After the treatment of A-PRF or HA + A-PRF for 0, 3 or 7 days, upper tips of fibrin gel were collected and processed for ELISA to detect VEGF according to Methods. *p<0.05, Mann-Whitney test.

Table 1. Δ VEGF concentrations of Fibrin Gels. VEGF concentrations of A-PRF or HA+A-PRF for 3 and 7 days were subtracted with the concentrations of A-PRF or HA+A-PRF for 0 day. The *p*-values were the results of Mann-Whitney test.

Δ Day	A-PRF (pg/mL)	HA + A-PRF (pg/mL)	*p*-value
0−3	142.87±115.37	728.87±311.98	0.000
0−7	299.04±281.77	923.85±419.71	0.002

PDGF Concentration in Fibrin Gel of A-PRF and HA + A-PRF Groups

PDGF concentration baseline (day-0) of A-PRF group (135.18±127.34 pg/mL) was lower than the PDGF concentration of HA + A-PRF group (164.48±153.66 pg/mL), but not significant (p=0.880, Mann-Whitney test) (Figure 2). On day-3, PDGF concentration of HA + A-PRF group (640.56±173.31 pg/mL) was significantly higher (*p*=0.019, Mann-Whitney test) than the one of A-PRF group (405.36±228.72 pg/mL). The PDGF concentration was also continuously and significantly increased on day-7 (*p*=0.004, Mann-Whitney test), the PDGF concentration of HA + A-PRF group (876.27±257.53 pg/mL) compared to the one of A-PRF group (479.57±236.58

pg/mL). Meanwhile, ΔPDGF concentration of HA + A-PRF group was also significantly higher than the one of A-PRF group for both Δ0-3 and Δ0-7 (Table 2).

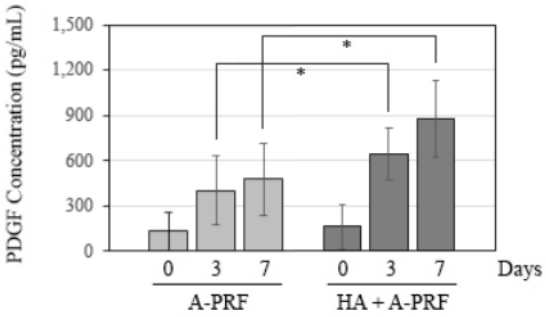

Figure 2. PDGF concentrations of Fibrin Gels. After the treatment of A-PRF or HA+A-PRF for 0, 3 or 7 days, upper tips of fibrin gel were collected and processed for ELISA to detect PDGF according to Methods. *$p<0.05$, Mann-Whitney test.

Table 2. Δ PDGF concentrations of Fibrin Gels. PDGF concentrations of A-PRF or HA+A-PRF for 3 and 7 days were subtracted with the concentrations of A-PRF or HA+A-PRF for 0 day. The *p*-values were the results of Independent Sample T test.

Δ Day	A-PRF (pg/mL)	HA + A-PRF (pg/mL)	*p*-value
Δ Day 0−3	270.19±174.57	476.08±181.94	0.019
Δ Day 0−7	344.39±292.66	711.79±328.50	0.017

DISCUSSION

Type 2 Diabetes Mellitus (T2DM) is often associated with chronic hyperglycemia which can lead to inhibition of wound healing. For DFU treatment, topical growth factors are important, since the chronic diabetes patients has a decrease growth factors and prolonged inflammation in which will inhibit healing.(17,18) In current study, the topical growth factor was provided from PRF obtained from the blood of the subjects (autologous).

Although several studies on the HA and PRF have been published, the effects of mixing PRF and HA have not been fully understood. PRF can stimulate the healing process of different tissues by delivering various growth factors and cytokines that are released by platelets.(19) In current study, adding HA to the PRF increased the concentration of VEGF and PDGF release on day-3 and day-7, which possibly could induce the effect of A-PRF on DFU.

Signal transduction plays important roles in inducing cell proliferation both in tissue regeneration and malignancy.(20-22) HA which is used in this study, could induce Cluster of Differentiation (CD)44 and Receptor for Hyaluronan Mediated Motility (RHAMM) for angiogenesis transduction in vascular endothelial cells.(23) RHAMM-ligand interaction of endothelial cells will increase endothelial cell motility, and CD44-ligand interaction increases endothelial cell proliferation.(24) Both CD44 and RHAMM work in tandem to facilitate formation of new blood vessels. HA also activates several CD44-dependent isoforms such as Protein Kinase C (PKC), Raf-1 kinase, Mitogen-activated Protein/Extracellular Signal-regulated Kinase Kinase (MEK)-1, and Extracellular Signal-regulated Kinase (ERK)1/2, so that it will increase endothelial cell proliferation.(25) In this study, compare to A-PRF merely, HA + A-PRF significantly increased PDGF release from fibrin gel on day-3 and day-7.

Diabetes in chronic hyperglycemia has reduced capacity in the proliferation and synthesis of collagen because it is unresponsive to transformations of growth factor-β1 (TGF-β1) stimulation. Either platelet rich fibrin (PRF)-lysate or hyaluronic acid (HA) can restore the TGF-β1 signaling pathway. Improving TGF-β1 signaling was measured by the cellular proliferation index and collagen deposition. The addition of HA to PRF-lysate resulted in a significant increase in the proliferation index and collagen deposition index rather than PRF-lysate alone. (26)

In an in vitro study, a mixture of PRF and 3% HA was reported to increase the release of TGF-β, PDGF-BB and FGF. In the combination HA + A-PRF, can cause the platelets to be more permeable, which triggers the release of growth factors by α-granules.(27)

CONCLUSION

The concentration of growth factors released by A-PRF on day-3 day-7, were increased by the addition of HA. Taken together, HA accelerates growth factor release from A-PRF. A mixture of A-PRF and HA could be more effective than A-PRF alone for treatment of DFU.

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AUTHORS CONTRIBUTION

RWK, IA, FDS, EY, and SB designed the study. RWK collected the study data. RWK, IA, FDS, FS, EY, and SB did the statistical analysis. RWK, IA, FDS, FS, EY, SI, TS, JR, SB, and MHR

interpreted the data. All authors contributed in preparing the manuscript. FDS and FS gave writing advice. FDS collected the study fund.

REFERENCES

1. Bhanot S, Alex JC. Current applications of platelet gels in facial plastic surgery. Facial Plast Surg. 2002; 18: 27-33.
2. Oktaviono YH, Susanti M, Lefi A, Sandra F. Human umbilical cord blood-derived secretome enhance endothelial progenitor cells migration on hyperglycemic conditions. Pharmacogn J. 2020; 12(4): 793-7.
3. Dewi DAR, Sandra F. Conditioned media of human umbilical cord blood mesenchymal stem cell inhibits ultraviolet B-induced apoptosis in fibroblasts. Indones Biomed J. 2019, 11(1): 85-90.
4. Whitman DH, Berry RL, Green DM. Platelet gel: an autologous alternative to fibrin glue with applications in oral and maxillofacial surgery. J Oral Maxillofac Surg. 1997; 55: 1294-9.
5. Soffer E, Ouhayoun JP, Anagnostou F. Fibrin sealants and platelet preparations in bone and periodontal healing. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2003; 95: 521-8.
6. Mazzucco L, Medici D, Serra M, Panizza R, Rivara G, Orecchia S, *et al*. The use of autologous platelet gel to treat difficult-toheal wounds: a pilot study. Transfusion. 2004; 44: 1013-8.
7. Borzini P, Mazzucco L. Platelet gels and releasates. Curr Opin Hematol. 2005; 12: 473-9.
8. Everts PA, Knape JT, Weibrich G, Schonberger JP, Hoffmann J, Overdevest EP, *et al*. Platelet-rich plasma and platelet gel: a review. J Extra Corpor Technol. 2006; 38: 174-87.

227 9. Anitua E, Andia I, Ardanza B, Nurden P, Nurden AT. Autologous platelets as a source of
228 proteins for healing and tissue regeneration. Thromb Haemost. 2004; 91: 4-15.
229 10. Lind M. Growth factor stimulation of bone healing. Effects on osteoblasts, osteomies, and
230 implants fixation. Acta Orthop Scand Suppl. 1998; 283: 2-37.
231 11. Kandziora F, Pflugmacher R, Scholz M, Schafer J, Schollmeier G, Schmidmaier G, *et al*.
232 Dose-dependent effects of combined IGF-I and TGF-beta1 application in a sheep cervical
233 spine fusion model. Eur Spine J. 2003; 12: 464-73.
234 12. Kartika RW, Alwi I, Suyatna FD, Yunir E, Waspadji S, Immanuel S, *et al*. Advance-platelet
235 rich fibrin and hyaluronic acid combination improves interleukin-6 and granulation index in
236 diabetic foot ulcer patients. Indones Biomed J. 2021; 13: 170-7.
237 13. Schmidt MB, Chen EH, Lynch SE. A review of the effects of insulin-like growth factor and
238 platelet derived growth factor on in vivo cartilage healing and repair. Osteoarthritis Cartilage.
239 2006; 14: 403-12.
240 14. Marx RE. Platelet-rich plasma: evidence to support its use. J Oral Maxillofac Surg. 2004; 62:
241 489-96.
242 15. Meilana A, Dewi NM, Wijaya A. Stem cell therapy in wound healing and tissue regeneration.
243 Indones Biomed J. 2016; 8: 61-70.
244 16. Levett PA, Hutmacher DW, Malda J. hyaluronic acid enhances the mechanical properties of
245 tissue-engineered cartilage constructs. PLoS One. 2014; 9: 11-22.
246 17. Azyenela R, Julianto I, Widodo Y, Wirohadidjojo. The addition of hyaluronic acid into
247 platelet-rich fibrin lysate in restoration of senescent human dermal fibroblasts activities.
248 Malays J Med Biol Res. 2016; 3: 1-8.

18. Sargowo D, Handaya AY, Widodo MA, Lyrawati D, Tjokroprawiro A. Aloe gel enhances angiogenesis in healing of diabetic wound. Indones Biomed J. 2011; 3: 204-15.
19. Choukroun J, Diss A, Simonpieri A, Girard MO, Schoeffler C, Dohan SL, *et al*. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part IV: clinical effects on tissue healing. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006; 101: 56-60.
20. Sandra F, Oktaviono YH, Widodo MA, Dirgantara Y, Chouw A, Sargowo D. Endothelial progenitor cells proliferated via MEK-dependent p42 MAPK signaling pathway. Mol Cell Biochem. 2015; 400(1-2): 201-6.
21. Oktaviono YH, Putri AY, Al-Farabi MJ, Firmansyah YR, Sandra F. Extracellular-Signal Regulated Kinase Signalling Pathway Mediates the Increased Proliferation of EPCs Treated with Garlic (Allium sativum) Extract, Purple Sweet Potato (Ipomoea batatas) Extract, and Vitamin C. Pharmacognosy Journal, 2020, 12(3): 442-7.
22. Hendarmin L, Sandra F, Nakao Y, Ohishi M, Nakamura N. TNFα played a role in induction of Akt and MAPK signals in ameloblastoma. Oral Oncol. 2005; 41(4): 375-82
23. Hall CL, Lange LA, Prober DA, Zhang S, Turley EA. pp60(c-src) is requiredfor cell locomotion regulated by the hyaluronanreceptor RHAMM. Oncogene. 1996 ;13: 2213-24.
24. Savani RC, Cao G, Pooler PM, Zaman A, Zhou Z, DeLisser HM. Differential involvement of the hyaluronan (HA) receptors CD44 and receptor for HA-mediated motility in endothelial cell function and angiogenesis. J Biol Chem. 2001; 276: 36770-8.
25. Slevin M, Krupinski J, Kumar S, Gaffney J.Angiogenic oligosaccharides ofhyaluronan induce protein tyrosine kinase activity in endothelial cells andactivate a cytoplasmic signal transduction pathway resulting in proliferation. Lab Invest. 1998; 78: 987-1003.

271 26. Hu Y, Wu Y, Cai J, Ma S, Wang X. The procoagulant properties of hyaluronic acid- collagen
272 (I)/chitosan complex film. J Biomater Sci Plym Ed. 2009; 20: 111-8.
273 27. Bourguignou LY, Singleton PA, Zhu H, Hyaluronan promotes signaling interaction between
274 CD44 and the transforming growth factor beta receptor I in metastatic breast tumor cells. J
275 Biol Chem. 2002; 277: 303-12.

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