Sys Rev Pharm 2020;11(12):519-526
A multifaceted review journal in the field of pharmacy

The Use of Image Processing in the Evaluation of Diabetic Foot Ulcer Granulation after Treatment with Advanced-Platelet Rich Fibrin + Hyaluronic Acid

Ronald W. Kartika[1], Idrus Alwi[2], Franciscus D. Suyatna [3], Em Yunir[2], Sarwono Waspadji[2], Suzzana Immanuel[4], Todung Silalahi [5], Saleha Sungkar[6], Jusuf Rachmat[7], Mirta Hediyati Reksodiputro [8], Saptawati Bardosono[9]*

[1]Doctoral Program in Medical Science Faculty of Medicine Universitas Indonesia
[2]Department of Internal Medicine, Faculty of Medicine Universitas Indonesia – Cipto Mangunkusumo Hospital, Jakarta, Indonesia
[3]Department of Clinical Pharmacology, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia
[4]Department of Clinical Pathology, Faculty of Medicine Universitas Indonesia – Cipto Mangunkusumo Hospital, Jakarta, Indonesia.
[5]Department of Internal Medicine, Krida Wacana Christian University, Jakarta, Indonesia.
[6]Department of Clinical Parasitology, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia
[7]Department of Thorcic Cardiac and Vascular Surgery, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia
[8]Facial Plastic Reconstructive Division, Department of Otorhinolaryngology, Faculty of Medicine, Universitas Indonesia, Cipto Mangunkusumo Hospital, Jakarta, Indonesia
[9]Department of Nutrition, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia (correspondence Author)
Emails: tati.bardo@yahoo.com

ABSTRACT

Introduction: It is difficult to objectively assess wound healing process as characteristics of different types of wounds vary markedly. Reliable and valid photograph wound assessment tools to assess wound appearance is critical to provide objective measurement for clinical trials.
Objective: This study aims to describe the use of digital image analysis system, ImageJ to objectively measure wound area, red granulation tissue, and granulation index for diabetic foot ulcer (DFU).
Methods: Images were taken from an open label randomized controlled trial of DFU patients with wound duration ≥ 3 months, Wagner-2 and ulcer size < 40 cm^2 at Koja and Gatot Soebroto Hospital, Jakarta, Indonesia from July 2019 to April 2020. The study was approved by the ethics committee of the Faculty of Medicine Universitas Indonesia ID 0855/UN2.F1/ETIK/2018. A total of thirty subjects were recruited, after receiving informed consent, and were randomized into three groups of ten, each group receiving different intervention of topical autologous platelet rich fibrin (A-PRF), A-PRF and Hyaluronic Acid (HA) or 0.9% NaCl solution (control). All patients underwent strict glycemic control and debridement. Images were taken at baseline, day 3, day 7 dan day 14. Wound area, granulation area, granulation index and their corresponding changes over time were measured with ImageJ software. Statistical analyses were performed using SPSS version 20.
Results: In A-PRF +HA group, there was no significant increase (p=0.597) of delta percentage in wound area on day-0 (8.4 cm^2) today-7 (35.8 cm^2) compared with PRF on day-0 (12.8 cm^2) today-7 (34.4 cm^2) and control on day-0 (14.6 cm^2) today-7 (34.7 cm^2) . Meanwhile, there was a significant increase (p=0.035) of delta percentage granulation area on day-0 (1.4 cm^2) today-7 (2.4 cm^2) compared with PRF on day-0 (0.4 cm^2) today-7 (1.0 cm^2) and control on day-0 (0.2 cm^2) today-7 (0.7 cm^2). There was also a significant increase (p=0.049) of delta percentage granulation index on day-0 (26.0%) to day-7 (57.7%) compared with PRF on day-0 (12.5% cm^2) today-7 (50.9%) and control on day-0 (12.8%) to day-7 (39.9%).
Conclusion: ImageJ provides a reliable and valid tool of measurement to assess wound healing process in diabetic foot ulcer use granulation area measurement and granulation index method.

Keywords: Diabetic foot ulcer, granulation index, wound healing, wound image

Correspondence:
Saptawati Bardosono
Department of Nutrition, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia
Emails: tati.bardo@yahoo.com

INTRODUCTION

Diabetic foot ulcers (DFUs) represent a huge challenge in today's healthcare system. Wound healing is a slow process and daily care is necessary. DFU in type 2 diabetes is a significant health problem affecting 5–6 million people in the United States (US).[1-4] DFUs are painful, prone to infection, and are difficult to heal, requiring both time and effort.[5] It is estimated that approximately 71,000 of lower limb amputations in 2004, are diabetes-related, making it the primary cause of non-traumatic lower limb amputations. The cornerstone of DFU management is daily care. Daily care improves wound healing and lowers the chance of infection, reducing the risk for amputation and other complication, and greatly reducing the massive healthcare costs for DFU and its complications.[6] Indicators of wound healing speed are the proportion of granulation tissue to the wound area, epithelial tissue, and complete closure.

Current standard DFU treatment is a combination of daily self-care and physician examination during regular visits to a wound clinic. The wound is examined by experienced physicians and the healing status compared to that of the previous visit. Various methods can be used to evaluate wound changes, but the most commonly used tool is planimetry, using the Grid method. The Grid method is accurate, objective, and inexpensive. However, it is time consuming to draw the wound and is limited to measurement of the area of the wound, but not the growth of granulation tissues.[7]

Thus, a wound measurement method was developed that allows evaluation of the granulation tissue using photography, which was then analyzed with digital wound analysis software.[8] The software can be used to measure wound area (WA), granulation tissue area (GA) and granulation index (GI). GI measurement was developed to analyze the earlier phase of wound healing - the proliferative phase and gives a predictor of good wound healing outcome.[9, 10]

This study aims to standardize usage of ImageJ software to analyze the wound area, granulation tissue and granulation index of DFU patients as an alternative to conventional Planimetry Grid system.

METHODS

Study Design

We conducted an open label, three arms, randomized controlled trial (RCT) from July 2019 to April 2020 in Koja District Hospital and Gatot Soebroto Army Hospital in Jakarta, Indonesia. Patients with DFU and an average wound duration of 3 months, categorized as Wagner-2, ulcer area <40 cm^2 were recruited into the study. Patients who were uncooperative, with severe anemia with hemoglobin (Hb) < 8,0 g/L, patients with very poor glycemic control (HbA1c >12,0%/108 mmol/mol), platelet *concentration* < 100 x 10^9/L, patients with severe chronic kidney disease on routine hemodialysis, patients with hematologic disorder such as sickle cell anemia or leukemia, patients with bleeding disorder such as hemophilia were excluded. Patients who fail to complete follow up were dropped out. A total of thirty patients were recruited, asked for informed consent and randomized equally into the three arms, with 10 subjects in each for DFU treatment with A-PRF+AH, A-PRF and control group. In each arm, treatment was administered on day 0, 3 dan 7. Photographs were taken at baseline, day 3, day 7 and day 14.

Ethical Approval

The study was approved by the Ethics Committee of the Faculty of Medicine of Universitas Indonesia ID 0855/UN2.F1/ ETIK/2018. Informed consent was obtained from the patients, including for the use of photographs.

Data processing

Photographs taken were processed with ImageJ software. Wound area (WA) was measured by tracking the edge of the wound in cm^2. Granulation area (GA) was assessed using color segmentation within the boundaries of the wound image using a color cluster algorithm based on the Red-Yellow-Black color distinction (Figure 1).[8] The area colored red represented the GA in cm^2. GI was calculated as the percentage of granulation area against the total wound area (Figure 2) using the formula as follows: (GA/WA) x 100%. Numerical data was then extracted from ImageJ. Statistical analysis was performed using SPSS version 20.

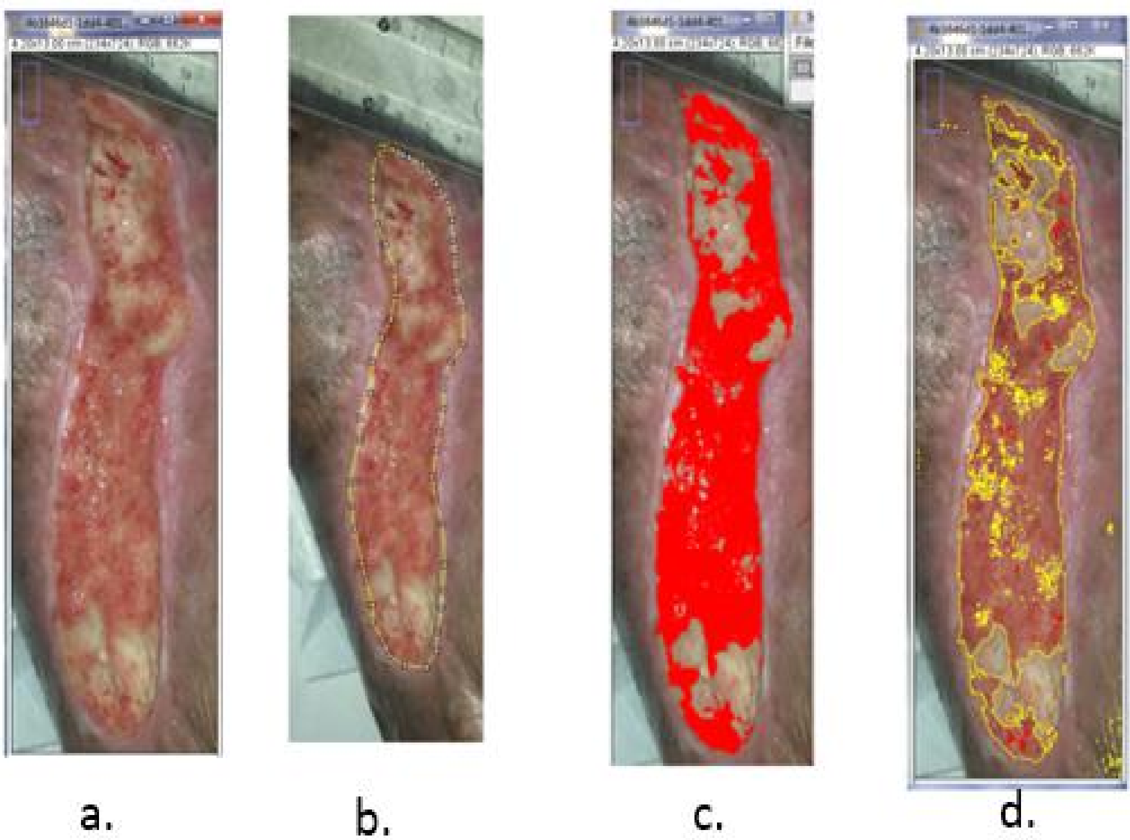

Figure 1. The ImageJ tracing and processing to quantify granulation tissue. A) Wound photograph of DFU patient B) Wound area tracing C) & D) Granulation tissue

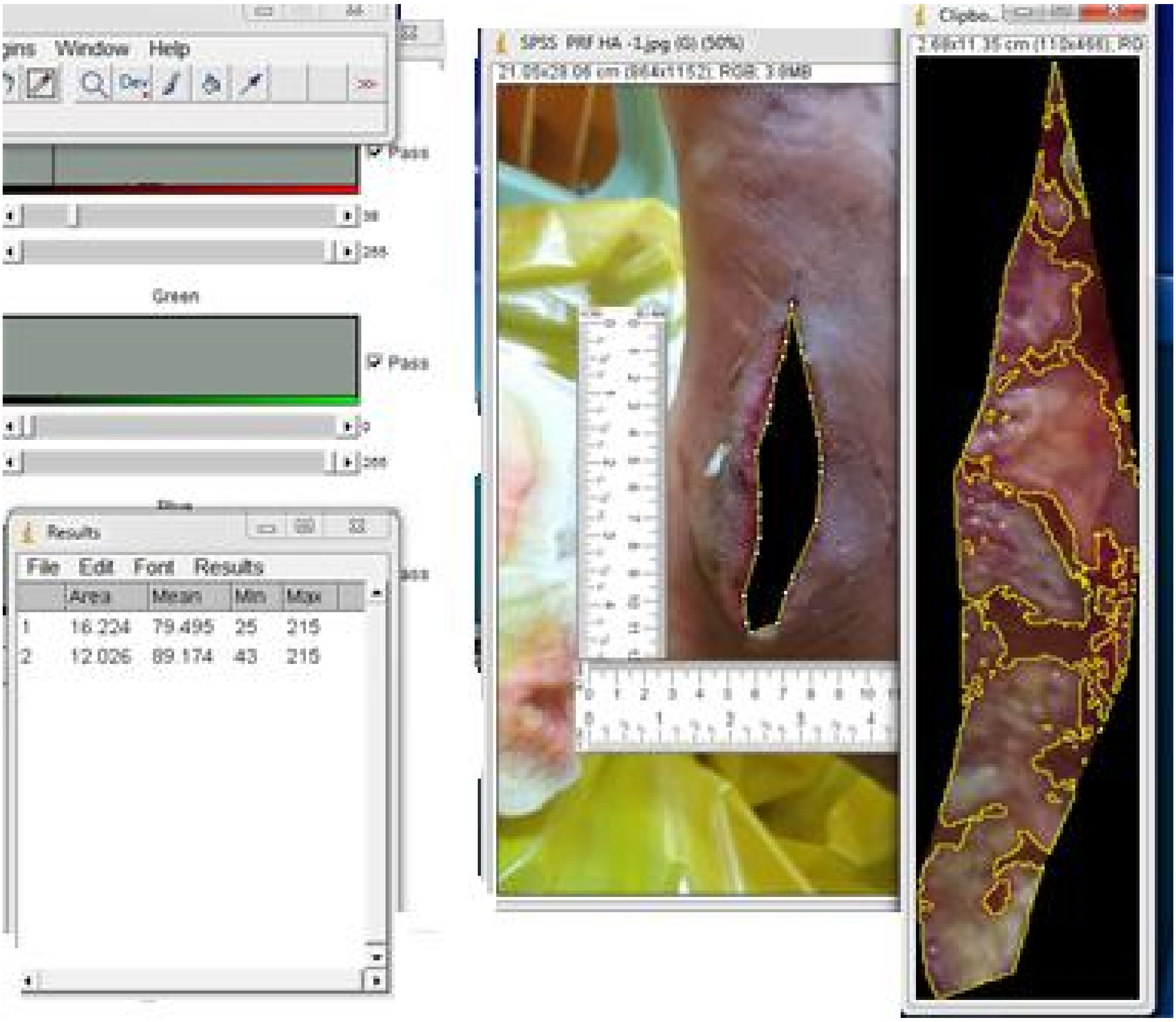

Figure 2. The measurement of granulation index using ImageJ processing software

RESULTS

A total of thirty subjects were recruited, from each subject, four photographs were taken at different time points. Photographs were processed and numerical data was obtained. The baseline characteristics of the subjects are presented in Table 1. Normal data is presented as mean ± standard deviation (SD) while non-normal data is presented as median (min-max).

Table 1. Baseline characteristics of subjects recruited into the study

Characteristics	A-PRF+AH (n = 10)	A-PRF (n = 10)	Control (n = 10)	p
Age (year)[a]	59.8 ± 12.7	64.7 ± 12.0	66 (36–71)	0.626[+]
Sex Male Female	 5/10 5/10	 4/10 6/10	 3/10 7/10	
BMI[a]	28.9 ± 2.7	27.3 ± 2.08	28.4 ± 2.5	0.337[+]
Hemoglobin (g/dL)	12.7 (27.4–39.0)	13.1 ± 1.3	12.05 (10.1–16.5)	0.224[+]

Hematocrit e(%)	36.3 (29.2–42.9)	35.6 ± 4.6	33.8 (24.4–40.8)	0.145⁺
Leukocyte (10^3/µl)	13.30 ± 1.08	11.08 ±1.33	9.23 ± 1.66	0.985*
Platelet (10^3/µl) Random Blood Glucose. mg/dL	354.9 ± 167.5 286.0 (170–390)	338.8 ± 164.5 243.8 ± 47.4	319.9 ± 128.4 254.7 ± 58.6	0.880* 0.104⁺
HbA1C (%) [a] Cholesterol total (mg/dL)	11.34 ± 1.30 214.5 ±16.9	9.0 ± 0.68 249.3 ± 16.1	8.5 ± 0.72 202.3 ± 38.6	0.950* 0.096*
Albumin (mg/dL)	3.3 (2.8–4.2)	3.1 (2.8–4.2)	3.2 ± 0.39	0.662⁺

*ANOVA, ⁺Kruskal Wallis

The average wound area, granulation area and granulation index were then calculated and shown in Table 2.

Table 2. Average wound area, granulation area and granulation index

Time	**A-PRF+HA**			**A-PRF**			**Control**		
	WA	**GA**	**GI**	**WA**	**GA**	**GI**	**WA**	**GA**	**GI**
Baseline	7.0(1.9-31.9)	2.1(0.79–14.2)	38.2±14.4	4.6(2.3–9.7)	1.8(0.3–9.2)	34.8±16.8	5.2(2.0–0.6)	1.2(0.6–9.7)	36.0±15.4
D-3	6.3(1.4–26.1)	2.8 (1.1–17.2)	64.2±13.4	3.9(1.6–7.3)	2.2(0.3–10.4)	47.3±17.9	3.2(1.9–8.4)	1.4(0.6–10.2)	48.7±14.2
D-7	5.5(1.1–25.4)	4.4 (1.2–17.4)	79.9±9.9	3.6(0.6–4.4)	2.7(0.4–11.4)	63.8±18.5	2,6(1.1–7.1)	1.5(0.8–12.0)	60.5±11.4
D-14	5.0(0.9–8.6)	4.8(1.21–18.4)	95.9±3.9	3.4(0.6–3.0)	3.1(0.5-11.8)	85.7±16.2	2.3(0.3–6.2)	1.8(0.9–12.3)	75.9±9.6

Figure 3. shows the measurement, clinical evaluation and clinical photographs taken at baseline, day 3, day 7 and day 14 on different treatment groups. Here, we observed different rate of wound closure and healing, especially at day 14 compared to control group.

Figure 3. DFU wound photographs of different treatment groups taken at various timepoints to show progression of wound healing prior to processing using ImageJ software

Figure 4. shows the granulation index all treatment group. A-PRF+HA showed greatest GI value compared to other intervention groups.

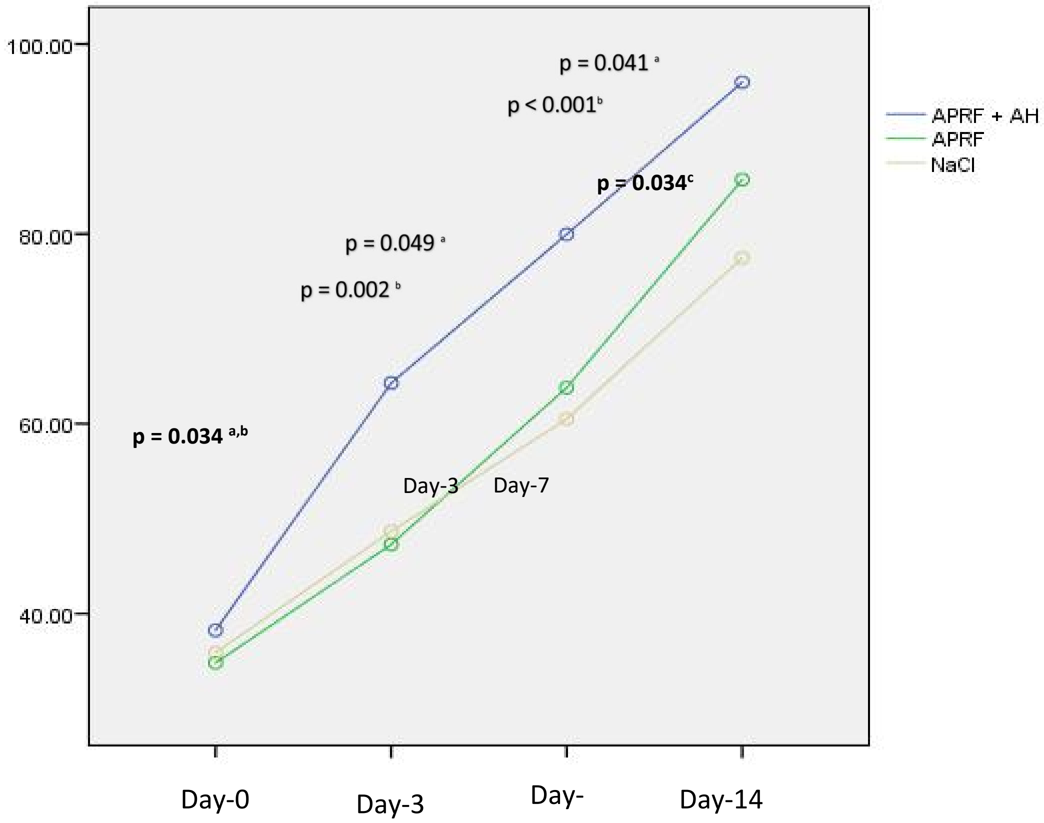

Figure 4. Granulation index for all treatment groups

[a] GI of A-PRF+HA group compare with A-PRF group, Mann Whitney test
[b] GI of A-PRF+AH group compare with control, Mann Whitney test
[c GI] of A-PRF group compare with control, Mann Whitney test

To demonstrate the speed of healing process, we then measured the difference in wound area, granulation area and granulation index at each time point compared to baseline (Δ %). These are shown in Table 3, 4 and 5 respectively. Table 3 shows the change in wound area on day-0, day-3, day-7 and day-14 showed no significant difference ($p > 0.05$) in all treatment groups.

Table 3. Δ%Wound Area of DFU in each treatment group

Change in WA	A-PRF+HA	A-PRF	Control	p value*
$\Delta\%^{D}3$	8.4 (1.0-57.1)	12.8 (0.78-37.1)	14.6 (3.47-44.6)	0.572
$\Delta\%^{D}7$	22.1 (3.48-44.7)	23.8 (7.22-72.7)	21.4 (10.4-77.1)	0.733
$\Delta\%^{D}14$	35.8 (11.8–45.5)	34.4 (11.6-77.3)	34.7 (19.0-94.8)	0.597

*Kruskal Wallis test

Table 4 shows the increase of granulation area in DFU patients for all treatment groups at different time points. There was a significant increase in granulation area on day-3 ($p = 0.009$), day-7 ($p = 0.025$) and day-14 ($p = 0.035$) compared to A-PRF and control.

Table 4. Δ% Granulation Area of DFU in each treatment group

Change in GA	A-PRF+HA	A-PRF	Control	p value*
$\Delta\%^{D}3$	1.4 (0.13-3.62)	0.4 (0.01-1.62)	0.2 (0.01-0.76)	0.009
$\Delta\%^{D}7$	2.1 (0.2-3.65)	0.8 (0.05-2.69)	0.4 (0.19-2.29)	0.025
$\Delta\%^{D}14$	2.4 (0.22-4.19)	1.0 (1.12-3.0)	0.7 (0.22-2.57)	0.035

*Kruskal Wallis test

Further analysis using Mann Whitney test showed significant differences in Δ% D3 between A-PRF +HA vs A-PRF ($p = 0.034$), A-PRF +HA vs control ($p = 0.003$) but not A-PRF vs control ($p = 0.406$). In Δ% D7, there were significant differences in A-PRF +HA vs A-PRF ($p = 0.041$), A-PRF +HA vs control ($p = 0.013$), but not A-PRF vs control

(p=0.450).In Δ% D14, only A-PRF +HA vs control (p =0,016) showed statistical significance while others did not.

Table 5 shows the Δgranulation index measurement. There were statistically significant differences in Δ granulation index on day-3, day-7 and day-14 ($p < 0.05$).

Table 5. ΔGranulation Index in each treatment group

Intervention	A-PRF+HA	A-PRF	Control	p value*
Δ % ^{D}3	26.0 (± 8.4)	12.5 (± 6.2)	12.8(± 5.1)	p < 0.001
Δ % ^{D}7	41.7 (± 13.8)	28.9 (± 9.2)	24.6 (± 8.8)	P = 0.004
Δ % ^{D}14	57.7 (± 14.1)	50.9 (± 17.6)	39.9 (± 14.5)	P = 0,049

*Anova test

Further post hoc analysis showed significant differences in Δ% D3 A-PRF +HA vs A-PRF (p < 0.001), A-PRF +HA vs control (p < 0.001), but not A-PRF vs control (p=1.000). In Δ% ^{D}7, there were significant differences in A-PRF +HA vs A-PRF (p = 0.042), A-PRF +HA vs control (p = 0.005), but not A-PRF vs control (p=1.000). In Δ% ^{D}14, only A-PRF +HA vs control (p = 0.048) showed statistical significance while others did not.

DISCUSSION

The assessment of wound healing is often subjective thus developing methods to objectively quantify the state of wound healing is critical for future interventional studies involving wound healing processes. Over the years, different methods have been used to assess wound healing including clinimetric, tracing the wound area, kordic measurement, planimetrics and grid method, most of which only measure the wound or volume area.[10] However, the progression of the healing process is also influenced by granulation formation before the wound close completely.

There are four phases in wound healing, namely hemostasis, inflammation, proliferation, and remodeling phase. In proliferative phase, the granulation tissue is vital for the formation of epithelial tissue which is later on needed in the phase of remodeling.[11] In DFU, epithelial tissues form at around 0,028 mm/day at the edge of DFU wound area. The formation of tissues then decreases over time, following the fall of growth factors and chronic inflammatory substances. This decline in the tissue formation is the main reason for the slow, and thus, long duration of wound healing. Thus, it is necessary to produce topical treatment to improve wound healing.[12] One of such therapies for DFUs is the combination of A-PRF and HA. Many of the clinical studies revolve around these adjuvants to induce faster growth and thus, accelerated progression of wound healing. Thus, the use of an objective method to quantify the state of wound healing progression becomes critical to ensure accuracy of these studies. In this study, the use of digital photograph and ImageJ processing software is the method the authors propose to objectively quantify the state of wound healing.

ImageJ Software

ImageJ software is a public domain Java-based image processing program developed by the National Institutes of Health, Rockville, MD (http://imagej.net/ImageJ). ImageJ measures wound area with excellent producibility and reliability. The way to calculate the wound area in DFU is by manually tracing the wound edges. This method has high sensitivity, but in a complete DFU healing process, it takes a very long time.[13]

An indicator of wound healing is the formation of granulation tissue. The wound was photo-graphed digitally and treated with the Image-J program. In the Image-J program. you can distinguish healthy tissue (red color) and less healthy tissue (blue color). The proportion of red to blue is a sign of wound healing.[14,15]

Wound Area Measurement

Margolis et al. used variables as a surrogate marker for total wound healing, such as percent change in wound area, log healing rate, and log area ratio at week 2 and end of treatment at week 5. Three different formulas were proposed, the use of percent change of area, log of the area or ratio of the log area, with formulas as shown below.

(1) $\text{Percent change area} = \frac{Area_0 - Area_t}{Area_0} \times 100$

or

(2) $\text{Log Healing Rate} = \frac{Area_0 - Area_t}{Area_0} \times 100$

or

(3) $\text{Ratio of Log area} = \frac{\ln(Area_0)}{\ln(Area_t)}$

This study used method number 1 to see the differences in the wound area. The result showed that there was no significant difference in changes in wound area with different therapies.[10,18]

Several studies have used changes in granulation tissue area for the development of wound development. Robert A et al.[10] used percent of granulation for wound healing.

$$\text{Percent of granulation} = \frac{\text{Area with granulation tissue}}{Area_0} \times 100\%$$

Wound and granulation area measured by planimetry by manual tracing on a transparent plastic grid sheet, with a validated portable wound measuring device.[10]

This study also measured changes in granulation tissue from day 3, 7 and 14 compared to granulation tissue before DFU treatment. Although there are closely different delta granulations on day 3, 7 and 14, it is not yet known whether the growth of granulation tissue is followed by a reduction in the area of the wound. The time for granulation tissue measurement was done before the end of the treatment, where in DFU patients it usually takes more than 1 month for the wound to completely close.[2,19]

For this reason, another method (GI), or index measurement of granulation was considered by comparing the area of granulation tissue with the area of the wound at the same time point.

The granulation index measurement method can be used to evaluate the progress of DFU healing as a surrogate in terms of the area of the wound and the extent of the granulation tissue. Granulation index observations were taken and calculated on day-3, day-7 and day-14 to see the progress of wound healing. The average of wound area in the form of median (min-max) on day-0, day-3, day-7 and day-14 showed no significant difference in all treatment groups. In this study, the wound area showed no significant decrease, otherwise this study focusses on granulation tissue formation. In DFU healing, the formation of granulation tissue is determined by growth factors and inflammatory status at the wound healing processing.[19] Zhou[17] reported that DFU requires > 4 weeks to completely heal due to gradual decrease in growth factor and prolonged inflammation.

In this study we did not find any significant decrease in wound area in day-3, day-7 and day-14. However, the study showed a significant decrease in delta granulation area of A-PRF+HA compared to A-PRF and control. In the brief observation provided by this study, we focused on the formation of granulation tissue which is the hallmark of the proliferative phase in wound healing process. While this does not show the complete picture of wound healing, assessment of granulation tissue formation is very useful, as it offers significant insight to physicians to determine whether the treatment effect is pointing towards the right direction.

CONCLUSION

ImageJ is one of the tools to measure wound area, granulation area and granulation index. ImageJ may be used to assess wound healing process by calculating granulation index on day-3 and day-7. At these time points, wound healing process has yet to complete but A-PRF+HA group clearly demonstrate the best progression compared to PRF and control group.

FUNDING SOURCES

Universitas Indonesia

CONFLICT OF INTEREST

This study was done as part of Ronald W Kartika's doctoral degree in Faculty of Medicine, Universitas Indonesia, Jakarta.

REFERENCES

1. Westerhof W. Leg Ulcers: Diagnosis and Treatment. Amsterdam, Netherlands: Elsevier Science; 1993.
2. Eriksson G, Eklund AE, Torlegard K, Dauphin E. Evaluation of leg ulcer treatment with stereophotogrammetry. Br J Dermatol 1979; 101:123-31.
3. Covington JS, Griffin JW, Mendius RK. Measurement of pressure ulcer volume using dental impression materials: suggestion from the field. Phys Ther 1989; 69:6904.
4. Pories WJ, Schaer EW, Jordan DR. The measurement of wound healing. Surgery 1966; 59:821-4.
5. Smith DJ, Bhat S, Bulgrin JP. Video image analysis of wound repair. Wounds 1992; 4:15.
6. Berg W, Traneroth C, Gunnarson A, Lossing C. A method for measuring pressure sores. Lancet 1990; 335:1445-6.
7. Joseph E Grey, Stuart Enoch, Keith G Harding. ABC of wound healing: wound assessment. BMJ 2006;332:(page).
8. Shaw J, Hughes CM, Lagan KM, Bell PM, Stevenson MR. An evaluation of three wound measurement techniques in diabetic foot wounds. Diabetes Care 2007;30(10):2641–2.
9. Plassmann P, Jones BF. Measuring leg ulcers by colour coded structured light. J Wound Care 1992; 1:35-8.
10. Warriner RA, Snyder RJ, Cardina MH. Differentiating diabetic foot ulcers that are unlikely to heal by 12 weeks following achieving 50% percent area reduction at 4 weeks. International Wound Journal (tahun);8(6):632-7.
11. Stotts NA. Seeing red, yellow and black: the three-color concept of wound care. Nursing 1990; 20:59-61.
12. Melhuish JM. Boardman M. Palmer K. Harding KG. (abstract). J Wound Repair Regeneration 1994; 1:68. cek lagi
13. Dinh T, Elder S, Veves A. Delayed wound healing in diabetes: considering future treatments. Diabetes Manage 2011;1(5):509–19.
14. Mekkes JR, Westerhof W, van Riet Paap E, et al. Evaluation of wound debridement using computerized image analysis. In: Westerhof W, Vanscheidt W, eds. Proteolytic Enzymes and Wound Healing. Heidelberg, Germany: Springer-Verlag, 1994.
15. Perednia D. What dermatologists should know about digital imaging. J Am Acad Dermatol l991; 25:89-108.
16. Margolis, Kantor J, David J, Efficacy and prognostic value of simple wound measurement. Arch Dermatol 1998; 134:1571-2.
17. Qi M, Zhou Q, Zeng W, et al. Growth factors in the pathogenesis of diabetic foot ulcers. Bioscience Landmark 2018; 23:310-317.
18. Van Riet Paap E, Mekkes JR, Estevez O, Westerhof W. A new color video image analysis system for the objective assessment of wound healing in secondary healing ulcers. Wounds 2017; 3:134-7.
19. Silve CM, Iglesia AD, Diaz JI, Martin A, Alos IB, Granulation Response and Partial Wound Closure Predict Healing in Clinical Trials on Advanced Diabetes Foot Ulcers Treated With Recombinant Human Epidermal Growth Factor.Diabetes Care 2013; 36(2): 210–215.