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Role of Autologous Platelet Derived Growth Factor and Fibrin Rich Plasma in Management of Chronic Non-healing Ulcer–A Pilot Study

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Authors' contributions

This work was carried out in collaboration between all authors. Authors PKP and VJ designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors PB, SP and OPT managed the literature searches and experimental process. Authors PKK, SP and PB managed the analyses of the study performed. All authors read and approved the final manuscript.

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ABSTRACT

Background and Objectives: Chronic non-healing ulcer (CNHU) develops due to infections, trauma or underlying any medical and surgical conditions. Ulcer that have failed to response all available mode of treatment for long duration are more likely to develop gangrene and infection prone to limb amputation. This is a major public health problem. None of the conventional treatments are anticipated to stimulate active wound healing. The objectives of this study is to test the efficacy of topically applied autologous platelet derived growth factors and fibrin rich plasma in active repair of chronic non healing ulcer.

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Methods: Patients having one or more ulcers who have been receiving conventional treatment for their at least for more than 6 months duration but showed no evidence of healing till date were included in this study. Total of 30 skin ulcers were registered, of which 15 patients did not return for follow up. Rest 15 patients were included as the study group. All ulcers were treated with autologous platelet derived growth factors and fibrin rich plasma enriched antibiotic ointment. We observed that 73.33% ulcers were complete healed & rest ulcers had signs of improvement.
Results: The study group showed complete healing in 73.33% ulcers and average 80% improvement observed in each ulcer, after applying autologous platelet derived growth factors and fibrin rich plasma. Significant ulcer healing was observed in patients who were less than 40 years of age, had no history of addiction for any toxic substance, and had ulcer size less than 30 sq cm. Ulcer healing rate was also found to be higher in cases whose duration of ulcer was within one year and those who did not have any history of systemic diseases.
Conclusion: This study clearly shows the efficacy of topically applied autologous platelet derived growth factors and fibrin rich plasma in management of chronic non-healing ulcer.

Keywords: Chronic non-healing ulcers; autologous platelet derived growth factors; fibrin rich plasma.

1. INTRODUCTION

Ulcers that have failed to proceed through an orderly and timely reparative process over a period of 3 months are known as chronic non-healing ulcer (CNHU) [1]. These ulcers were unable to produce satisfactory anatomic and functional integrity even after treatment with all available modalities.

Chronic wounds are common and ingenerate significant burden on health care services.

Nearly 6 million people suffer from chronic wounds in United States (US) with a rough prevalence of 2% of the general population [2,3]. The estimated prevalence of chronic ulcers in Indian population as depicted by Shukla et al. [4] in their study, was about 4.5 per 1000 population. They also found the incidence of acute wound to be more than double at 10.5 per 1000 population.

A recent study had revealed that in a course of life time, almost 10% of the population will develop a chronic wound, with a wound related mortality rate of 2.5% [5,6].

Vowden K et al. [7] in his study depicted a prevalence of 3.55per1000 population in United Kingdom (UK) with a cost burden of around 2.03 million pounds per 100,000 population.

As per the Wound healing Society, chronic wounds are classified into four categories: venous ulcers, pressure ulcers, diabetic ulcer and arterial insufficiency ulcer [8]. They are not limited to these aetiological factors. Most of the wound share common features of excessive inflammation, inability of dermal cells to respond to the reparative stimuli, cell-cycle dysfunction, changes in enzyme activities. But yet, the underlying patho-physiological derangements varies from ulcer to ulcer [9]. Aetiological factors like venous ulcers, vasculitis, trauma, communicable and non-communicable diseases have attributed to the aetiology of chronic wounds in Indian scenario. The principal systemic conditions that favors chronicity of wounds are like diabetes mellitus, atherosclerosis, tuberculosis, leprosy and even filariasis [4,5,10].

In US also diabetic ulcers and pressure ulcers are growing at double digit rates and 14-24% of diabetic foot ulcers end up with amputation [2,11]. A longer life expectancy along with the increasing trend of non-communicable diseases, the prevalence of chronic wounds is also likely to rise.

An interdisciplinary approach to systemic assessment and innovation of an optimal treatment strategy, are essential requisite to reverse the rising trend in wound chronicity and morbidity associated with it.

Wound healing practices has started since the time of Smith Papyrus in 1700 BC. But the revolutionary era of wound biology has embarked in 1962 when the first growth factor-epidermal growth factor was discovered. Since then, role of many growth factors in wound repair and regeneration have been studied. Focusing on such novel growth factors in wound healing will not only improvise human health but also reduce the healthcare cost.

Platelet derived growth factor is currently the only growth factor approved for treatment of chronic non healing ulcers [12,13]. Platelet derived healing factors are polypeptides produced in the platelets and are stored in the intracellular α-granules. These factors are released when the platelets are activated by thrombin and act in a paracrine fashion. They are potent mitogens for smooth muscle cells, capillary endothelial cells and fibroblasts, and thus promote neo-vascularization. Being a chemo-attractant for neutrophils and fibroblasts, these factors enable wound repair through fibroblast proliferation and collagen synthesis. These in vivo properties suggest that PDGF, derived from platelets at the site of injury, may play an important role in the initiation of repair process of wounds like deposition of neo-vascularised collagen mesh, granulation tissue formation and epithelialization. [9,13,14]

Knighton et al. [15] in 1986, showed accelerated epithelialization and 100% healing of chronic non-healing ulcers by the use of autologous platelet derived wound healing factors (PDWHF). This was the first ever clinical demonstration of locally acting growth factors derived from autologous platelets that promote the healing of chronic cutaneous ulcers.

Steed et al had also reported significantly improved ulcer healing rates in patients receiving topical platelet derived growth factor versus a placebo group [16].

Saad Setta et al studied the healing effect of platelet releasate on the healing of chronic diabetic ulcers and compared it to that with platelet-poor plasma. They concluded that platelet-rich plasma (PRP) significantly enhanced the healing rate in of chronic diabetic ulcers [17].

In this study we tested the efficacy of topically applied autologous platelet derived growth factors and fibrin rich plasma in the repair of CNHU. Our aim was to establish certain guidelines on the use of platelet derived growth factors and fibrin rich plasma in management of CNHU. Our treatment protocol utilizes the fundamental treatment principles for the management of non-healing ulcers and can prove to be highly relevant in day to day practice as it is quite risk-free and easy on the pocket.

2. MATERIALS AND METHODS

This was a pilot study on a minimum of 15 patients/ulcers attending OPD of Dr. BRAM Hospital Raipur, Chhattisgarh. The study was conducted in the Department of Biochemistry, Pt J.N.M. Medical College Raipur, Chhattisgarh.

The Local Ethical Committee and Institutional Stem Cell Committee had provided clearance for this study on 15 patients. All patients were insured during this study.

13 patients were registered for the study. Of them, two patients had two ulcers each. So the number of chronic non-healing ulcers enrolled for the study was 15.

2.1 Selection Criteria

Ulcers formed due to any cause that fulfills the following criteria were selected for the study.

1. Duration of ulcer should be more than 6 months.
2. Age of the patients should be at least 18 years of age (arbitrarily taken for better patient compliance)
3. Patients should have a normal platelet count (more than 150000/ cumm)
4. There should be no documented malignant changes in the histopathological slide of the ulcer site biopsy.
5. The patients must have failed to respond to all the available modalities for treatment of ulcers
6. Presence of any chronic diseases like diabetes mellitus, hypertension, obesity, malnutrition, malignancy, sickle cell disease, varicose vein, organ failure etc.
7. History of infectious diseases like Leprosy, Tuberculosis in the past but the patient should have received complete treatment for the same

2.2 Rejection Criteria

1. Any ulcer of less than 6 months duration.
2. If the patients age is within 18 years of age.
3. Patients with less platelet count (less than 150000/ cumm)
4. Presence of any active systemic infection or infection at the site of ulcer

2.3 Methods

After taking informed consent from patients, 20 ml of patient's venous blood was drawn in plain sterile tube under aseptic condition. It was centrifuged at 2500 rpm at room temperature for

12 minutes without any interval. Fibrin clot was removed from the junction of packed red cells and plasma. The fibrin clot was mixed with 30 grams antibiotic ointment (Soframycin). This ointment was dispensed in a sterile container. The patients were advised to apply the ointment locally three times per day after cleaning the wound with normal saline. Patients were advised not to clean the wound with hydrogen peroxide. Of course, unhealthy wounds were debrided and cleaned once or twice with hydrogen peroxide in the hospital itself.

The study population was divided into different groups to study the effect of the following factors on ulcer healing:

i. Age of the patient-

   a. Less than 40 years (n=7).
   b. More than 40 years (n=8)

ii. Addiction-

   a. Presence of some sort of addiction (n=3)
   b. Absence of any sort of addiction (n=8)

iii. Duration of ulcer-

   a. Less than 1 year (n=7)
   b. More than 1 year (n=8)

iv. Size of ulcer –

   a. Less than 30 sq.cm (n=13)
   b. More than 30 sq.cm (n=2)

v. Associated diseases-

   a. Presence of or previous history of diseases (n=8)
   b. Absence of associated diseases (n=7)

The patients were followed up every 15 days till healing is complete or at least for 6 months.

The clinical photographs have been taken during each visit for each of the patients compare the healing of the ulcers.

Data analysis was done using Chi Square to find the duration of healing and the percentage of conversion of non-healing ulcer to healing ulcer in the different groups. A 'p' value <0.05 was regarded as significant.

3. RESULTS

Table 1 depicts the effects of different parameters on healing of chronic ulcers. Significant healing was documented in patients with ulcer size less than 30 sq.cm, and those without any type of addiction.

In Table 2, the effect of different parameters on the rate of healing has been shown. It reveals that the number of patient who could achieve complete healing within the first follow-up, are significantly more when compared to their respective counter-group.

4. DISCUSSION

Our study comprised of a total of 15 ulcers from 13 different patients diagnosed to have CNHU.

Table 1. Effect of different parameters on ulcer healing

Parameters	Groups	Percentage of ulcers completely healed (n=11)	Percentage of ulcers partially healed (n=4)	Chi Sq. P value
Age	Less than 40 years	55%	25%	$x^2 = 1.093$
	More than 40 years	45%	75%	>0.05
Addiction	Present	27%	75%	$x^2 = 4.356$
	Absent	**73%**	25%	<0.05
Duration of ulcer	Less than 1 year	55%	25%	$x^2 = 1.093$
	More than 1 year	45%	75%	>0.05
Size of ulcer	Ulcer area less than 30 sq.cm.	**91%**	75%	$x^2 = 19.584$ <0.001
	Ulcer area more than 30 sq.cm.	09%	25%	
Association of systemic disease	Present	45%	75%	$x^2 = 1.224$
	Absent	55%	25%	>0.05

n=number of ulcers

Table 2. Effect of different parameters on rate of ulcer healing

Parameters	Groups	Percentage of ulcers completely healed within 1st follow-up (n=6)	P value
Age	Less than 40 years	83%	< 0.05
	More than 40 years	17%	
Addiction	Present	17%	<0.05
	Absent	83%	
Duration of ulcer	Less than 1 year	67%	>0.05
	More than 1 year	33%	
Size of ulcer	Ulcer area less than 30 sq.cm.	100%	<0.001
	Ulcer area more than 30 sq.cm.	None	
Association of systemic disease	Present	33%	>0.05
	Absent	67%	

n = number of ulcers those completely got healed within first follow-up (one week)

Two of these patients had two ulcers at two different sites and hence a total of 15 ulcers were included for the study. 73% of the total ulcers healed completely while the rest healed partially. The effects of different factors affecting ulcer healing have been represented in Table 1.

Of the ulcers those accomplished complete healing, 55 % of them achieved the same within the first follow-up period of one week. The effects of various factors on the rate of ulcer healing have been depicted in Table 2.

Increased age is a major risk factor for impaired wound healing. Many clinical and animal studies at the cellular & molecular level have depicted age related changes in microcirculation and delay in wound healing [18-20]. We observed that ulcers of 75% patients of more than 40 years of age, could not accomplish complete healing whereas 83% of younger study group displayed a significantly faster healing rate ($p<0.05$). Their ulcers got completely healed within the first follow-up period when compared to older group who required more than one follow-up in 80% cases. Aging is associated with altered inflammatory response such as delayed T cell infiltration in to the wound area with alteration in chemokine production and reduced macrophage phagocytic capacity. These entail in delayed re-epithelialization, angiogenesis & collagen deposition, thus chronic non-healing ulcers. Reduced collagen turn over, remodeling & decreased wound strength have been observed in aged mice as compared with young mice. Exercise has been reported to improve cutaneous wound healing in older adults by increasing the blood flow to the site [21,22].

Retarded healing is strongly associated with exposure to cigarette smoke & tobacco. Nicotine has been identified as a potent vasoconstrictor that hampers blood flow reaching the areas undergoing regeneration [23]. In concurrence to this fact, our study population also revealed incomplete healing in 75% cases that were exposed to some sort of addiction when compared to patients without any addiction ($p<0.05$). Besides altered wound healing, smokers also evidenced an increase in a variety of complications such as infection, wound rupture, anostomotic leakage, wound and flap necrosis, epidermolysis and reduced tensile strength of wounds [24,25].

The healing rate was again found to be significantly high in non-addicted cases. About 83% of them accomplished complete healing by the first follow-up in comparison to the addicted cases who achieved the same in only 17% cases ($p<0.05$). Clinical evidence and animal experiments have also suggested findings relevant to alcohol induced immune-modulation and its consequences on host innate and adaptive immune responses. This resulted in diminished host resistance against microbial pathogens and host defense [23,26,27]. Connective tissue restoration is also influenced by alcohol intake, and results in decreased collagen content, and alterations in the protease balance at the wound site [28]. Even acute ethanol-intoxication remarkably impairs wound vascularity and precipitate increased wound hypoxia and oxidative stress [29].

Wound duration is a recognized indicator for potentially slow healing in a variety of wound types. When the effect of duration of ulcer (since the time it has appeared at that site) on its healing was analyzed, 75% of the partially healed group consisted of ulcer of longer duration. The study group revealed faster healing

rate in those with short duration of ulcer history. 67% subjects accomplished healing within first follow-up (Table 2). Margolis et al. [30] had also correlated delayed healing with specific wound characteristics like large wound area, an ulcer of long duration and a reduced ankle-brachial pressure index. This could be due to decrease protease activity, development of senescent cell population that precede to release pro-inflammatory cytokines, impairs vascular supply and accelerate telomere degradation [31,32].

Several classification systems for diabetic foot ulceration have been devised to allow risk stratification. One such validated system is the S(AD)SAD classification system- Size (Area & Depth), Sepsis, Arteriopathy and Denervation- which identifies cross-sectional area and depth of the ulcer as important factors associated with ulcer healing [33,34]. In this system lower grading is associated with rapid healing.

Similarly, our study group also depicted a significant link between the size of ulcer on its wound healing. Of the completely healed ulcers, around 91 % cases had wound size of less than 30 sq.cm ($p<0.001$). It was also noteworthy that of the patients with lesser surface area of ulcer, 100 % of them were healed completely within the first follow up (Fig. 1) when compared to those ulcers with a greater surface area (Fig. 2). The ulcers with larger surface area required more than one follow-up for healing. Kramer and Kearney have also indicated the size and depth of ulcers as good predictors for healing [35]. It has been suggested that large size ulcers provide greater surface area for various infections and thus there is a tendency for exaggerated inflammatory response that impede tissue repair, epithelialization and angiogenesis at the site [30,31].

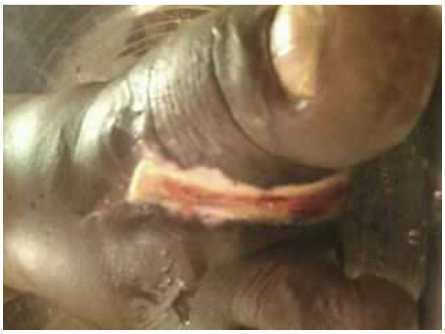

Fig. 1a- Initial wound size was 7x1-cm. between great toe and middle finger due to trauma

Fig. 1b- after treatment started wound completely healed with in 15 days

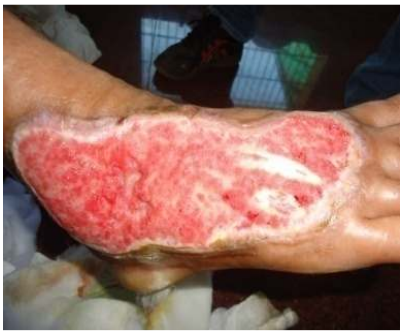

Fig. 2a- the initial wound size was 25x10 cm and tendon was also exposed

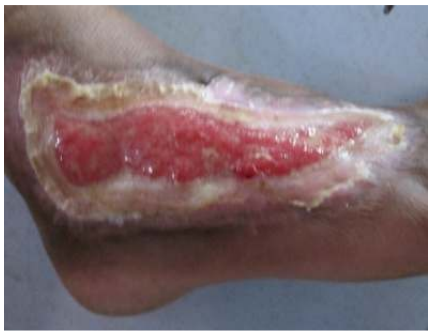

Fig. 2b- after treatment started 40% healing occurs within 15 days size was 18x8.2 cm

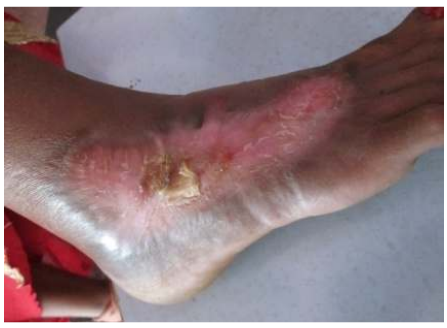

Fig. 2c- there was 100% healing in 30 days

Associated systemic disease is considered as a significant contributor for prolonged ulcer healing. 55% of ulcer patients that revealed complete healing were not associated with any evident systemic diseases. Of them 67% ulcers healed up completely within first follow-up. Treatment of chronic diseases like diabetes mellitus, obesity, malnutrition, peripheral vascular disease, malignancy, organ failure, sepsis plays a central role in wound management. Failure to attain optimum treatment of associated conditions will end up in chronic non-healing ulcers [23,31,36].

5. CONCLUSION

Autologous platelet derived growth factors and fibrin rich plasma promotes healing of chronic non-healing ulcers and leads to complete healing in about 74% ulcers in our study and others have also showed improvement. In the total ulcers, 40% ulcers were healed within 15 days. The duration of healing process depends on the age of patients, duration of ulcers, size of ulcer, addiction for alcohol and tobacco and presence of systemic disease or past history of systemic disease in the patients. There is no evidence of over healing, such as hypertrophy or keloid formation. The patient can apply medication at home without any difficulty. Patients feel benefitted as they don't have to undergo any surgery, only periodic out-patient examination is required and healing is achieved with little penny expenditure. However, to establish the validity of this hypothesis, a larger study group need to be recruited and necessitate more such studies.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Barbul A, Efron DT. Wound healing. Schwartz's principle of surgery, Mc Graw Hill companies. 9th edition, 2010;chapter-9:209-233.
2. Sen CK, Gordillo GM, Roy S, Kirsner R, Lambert L, Hunt TH, et al. Human skin wounds. Wound Repair Regen. 2009; 17(6):763-771.
3. Fife CE, Carter MJ, Walker D, Thomson B. Wound care outcomes and associated cost among patients treated in US outpatient wound centers: Data from the US wound registry. Wounds. 2012;24(1):10-17.
4. Shukla VK, Ansari MA, Gupta SK. Wound healing research: A perspective from India. International Journal of Lower Extremity Wounds 2005;4(1):7-8.
5. Sasanka CS. Venous ulcers of the lower limb: Where do we stand? Indian Journal of Plastic Surgery. 2012;45(2):266-274.
6. Agale SV. Chronic leg ulcers: Epidemiology, aetiopathogenesis and management. Ulcers. 2013:1-9. DOI: 10.1155/2013/413604
7. Vowden K, Vowden P, Posnett J. The resource costs of wound care in Bradford and Airedale primary care trust in the UK. J Wound Care. 2009;18:93-4.
8. The Wound Healing Society. Chronic wound care guidelines; 2006. Available:http://www.woundheal.org/assets/documents/final%20pocket%20guide%20treatment.pdf
9. Frykberg RG, Banks J. Challenges in the treatment of chronic wounds. Adv Wound Care (New Rochelle). 2015;4(9):560-582.
10. McDonald J. Global initiative for wound and lymphoedema care (GIWLC). Journal of Lymphoedema. 2009;4(2):92-95.
11. Kirketerp-Moller K, Zulkowski K, James G. Chronic wound colonization, infection and biofilms. Biofilm Infections, Springer Science+Business Media, LLC. 2011;11-24.
12. Chin KY, Anandan SM, Koshal K, Gujadhur P. Current and future developments in the treatment of chronic wounds. Open Access Surgery. 2013;6:43-53.
13. Kaltalioglu K, Coskun-Cevher S. A bioactive molecule in a complex wound healing process: Platelet-derived growth factor. Int J Dermatol. 2015;54(8):972-7.
14. Velnar T, Bailey T, Smrkolj V. The wound healing process: An overview of the cellular and molecular mechanisms. The Journal of International Medical Research. 2009;37:1528-1542.
15. Knighton DR, Ciresi KF, Fiegel VD, Austin LL, Butler EL. Classification and treatment of chronic nonhealing wounds. Successful treatment with autologous platelet derived wound healing factors (PDWHF). Ann Surg. 1986;204(3):322-330.
16. Steed DL. Clinical evaluation of recombinant human platelet-derived growth factor for the treatment of lower extremity diabetic ulcers. J Vasc Surg. 1995;2:71-81.
17. Saad-Setta H, Elshahat A, Elsherbiny K, et al. Platelet-rich plasma versus platelet-

poor plasma in the management of chronic diabetic foot ulcers: A comparative study. Int Wound J. 2011;8(3):307-12.
18. Gosain, DiPietro. Aging and wound healing. World J Surg. 2004;28:321-326.
19. Bielefeld KA, Amini-Nik S, Alman BA. Cutaneous wound healing: Recruiting developmental pathways for regeneration. Cell Mol Life Sci. 2013;70(12):2059-2081.
20. Bentov I, Reed MJ. The effect of aging on the cutaneous microvasculature. Microvasc Res. 2015;100:25-31.
21. Pence BD, Woods JA. Exercise, obesity and cutaneous wound healing: evidence from rodents and human studies. Adv Wound Care (New Rochelle). 2014;3(1): 71-79.
22. Emery CF, Kiecolt-Glaser JK, Glaser R, Malarkey WB, Frid DJ. Exercise accelerates wound healing among healthy older adults; A preliminary investigation. J Gerontol Med Sci. 2005;60(A):1432-1436.
23. Guo S, DiPietro LA. Factors affecting wound healing. J Dent Res. 2010;89(3): 219-229.
24. Chan LK, Withey S, Butler PE. Smoking and wound healing problems in reduction mammaplasty; is the introduction of urine nicotine testing justified? Ann Plast Surg. 2006;56:111-115.
25. Ahn C, Mulligan P, Salcido RS. Smoking-the bane of wound healing; Biomedical intervention and social influences. Adv Skin Wound Care. 2008;21:227-238.
26. Szabo G, Mandrekar P. A recent perspective on alcohol, immunity and host defense. Alcohal Clin Exp Res. 2009;33: 220-232.
27. Choudhry MA, Chaudry IH. Alcohol intoxication and post burn complications. Front Biosci. 2006;11:998-1005.
28. Radek KA, Kovacs EJ, Gallo RL, Dipietro LA. Matrix proteolytic activity during wound healing modulation by acute ethanol exposure. Alcohal Clin Exp Res. 2007;31: 1045-1052.
29. Radek KA, Kovacs EJ, Gallo RL, Dipietro LA. Acute ethanol exposure disrupts VEGF rceptor cell signelling in endothelial cells. Am J Physiol Heart Circ Physiol. 2008; 295:H174-H184.
30. Margolis DJ, Allen-Taylor L, Hoffstad O, Berlin JA. The accuracy of venous leg ulcer prognostic models in a wound care system. Wound Repair Regen. 2004;12: 163-8.
31. Vowden P. Hard-to-heal wounds: Made easy. Wounds International. 2011;2(4):1-6.
32. Bosanquet DC, Harding KG. Wound duration and healing rates: Cause or effect? Wound Repair Regen. 2014;22(2): 143-50.
33. Treece KA, Macfarlane RM, Pound N, et al. Validation of a system of foot ulcer classification in diabetes mellitus. Diabet Med. 2004;21(9):987-91.
34. Parisi MCR, Zantut-Wittmann DE, Pavin EJ, Machado H, Nery M, Jeffcoate WJ. Comparison of three systems of classification in predicting the outcome of diabetic foot ulcers in a Brazilian population. 2008;159:417-422.
35. Kramer JD, Kearney M. patient wound and treatment characteristics associated with healing in pressure ulcers. Adv Skin Wound Care. 2000;13(1):17-24.
36. Lantis JC 2nd, Marston WA, Farber A, Kirsner RS, Zhang Y, Lee TD, et al. The influence of patient and wound variables on healing of venous leg ulcers in a randomized controlled trial of growth-arrested allogenic keratinocytes and fibroblasts. J Vasc Surg. 2013;58(2):433-9.

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