Molecular and histopathological complications of wound healing in diabetic patients

Hadeel Khalaf Alboaklah Hadeel.khalaf@uokerbala.edu.iq

Department of Clinical Laboratory Science, College of Pharmacy, University of Kerbala

*Corresponding author at Tel: 009647724414265

Received [04/06/2021], Accepted [15/06/2021]

Abstract

Repair and tissue remodel procedures containing the chain of molecular and cytological actions that happen after any start of a cells injury to repair injured cells. The exudative, fibro multiplying, and extracellular atmosphere modifying stages are serial proceedings that arise via the combination of vital routes including solvable mediators, both parenchymal and hematocytes. Exudative occurrences that found after damage have a role in the expansion of tissue oedema. The fibroproliferative phase required to decrease the zone of connective tissue wound by contracting myofibril and fibroplasia. During this period, neovascularization developments will be detected. Endothelial cells are capable to convert into mesenchymal modules, and this modification looks to stand superbly arranged via a package of signalling proteins deliberate. This mechanism is called a Hedgehog. This review purposes to describe the numerous cytological, molecular features and histomorphological intricacies in the wound healing progression with diabetic patients.

Keywords: wound healing, tissue remodelling, Diabetic foot ulcers

Conflict of interest statement

The author state that he does not have a conflict of interest related to this review.

مضاعفات الجزيئية والنسيجية لالتئام الجروح لدى مرضى السكري

هديل خلف البوكلة

قسم علوم المختبرات السريريه ، كلية الصيدلة ، جامعة كربلاء

الخلاصه

عمليه التام الجروح واعاده ترميم الانسجه تتضمن سلسه من النشاطات التي تقوم بها الجزيئات الخلويه التي تحدث منذ بدايه حدوث الجرح الى مرحله الالتام. هناك مجموعه من العمليات المتسلسله خلال عمليه الالتام والتي تتضمن تكوين النضحه والتضاعف الليفي بالاضافه الى التغيير في المحيط الفسولوجي لمنطقه الجرح. عمليه غلق الجرح تتضمن اجتماع عدد من العمليات التي تتضمن ذوبان المواد الصلده في الخلايا الدمويه والخلايا الليفيه. اما مرحله التكاثر الليفي ضروريه جدا لتقليل مساحه النسيج الضام في الجرح وذلك بتقليص الليفات اللحميه وعمليه تضخم الييف العضلي وخلال هذه العمليه تبدا نشؤ اوعيه دمويه جديده والخلايا البطانيه تتحول الى عقد ميزنكيميه وهذه العمليات تنظم من خلال البروتينات الخلويه وهذه العمليه تدعى Hedgehogالقنفذ. هذه المقال تهدف الى وصف عدد من العمليات الخلويه الجزيئيه والتغيرات النسيجيه في عمليه التام الجروح لدى الأشخاص المصابين بالسكري.

Introduction

Pathogenesis of diabetic wound healing

Skin wound remedial is a vital biological progression involving the association of numerous cell types and their produces [1]. Motivations to repair the wound persuaded by limited aggression initiate very quick in the inflammatory phase [1]. At the end of the process, resulting in healing that contains the replacement of specific structures comes from the accumulations of the protein collagen, and activation, which relates to the development of cells multiplying and the following variation via previous cells in the injured tissue and bone marrow cells. These pathways not commonly disregard themselves, because after a skin cut, in the tissue, remodelling and repair can happen, the subject on the cell-damaging compromised by the wound [2]. Tissue redevelopment and healing event occur because of the trauma or subsequent from a particular pathological situation. the lesion is formed by all of the inducements that disrupt the physical stability of functional tissues. This inducement of lesions may be internal or external, as well as physical and chemical. Furthermore, the lesions can lead to harm to exact organelles or whole cells [3]. wound repair is a linear development in term of growth factors

(GF) seeking a cell proliferation, consequently directed to a combination of active modifications that implicate solvable mediators. [4]. Biochemical activities in damaged healing can be classified into the subsequent periods: inflammatory response, cell propagation and production of the factors which formed the extracellular background, and the later stage, named remodelling (Figure 1). These stages are not equally eliminating, but moderately overlap beyond time [5]. The skin ulcer in the foot area is a primary reason for hospitals admissions for diabetic patients in the entire world (Figure1).

A diabetic foot ulcer is a mutual of the supreme severe difficulties of diabetes-related to many occurrence and weakening of wound healthy enclosure. Imperfections in the vast circulation, which are frequently conducted by peripheral nerve and dysfunction of vessels, leading to the expansion of foot complications in diabetic patients. In addition, numerous structural skin foot distortions, which damage loading diagonally the surface, also related to weakened injure repairing of diabetic patients. Furthermore, diabetes modifies immune reaction and drops fighting to infection. Therefore, diabetic wound contamination further disruptions healing via numerous mechanisms [6]. In diabetic people, the wound remedial process is blocked by hyperglycaemia, prolonged inflammation, circulation disorders, hypoxia, nerve fibbers dysfunction and deteriorated neuropeptide signalling.

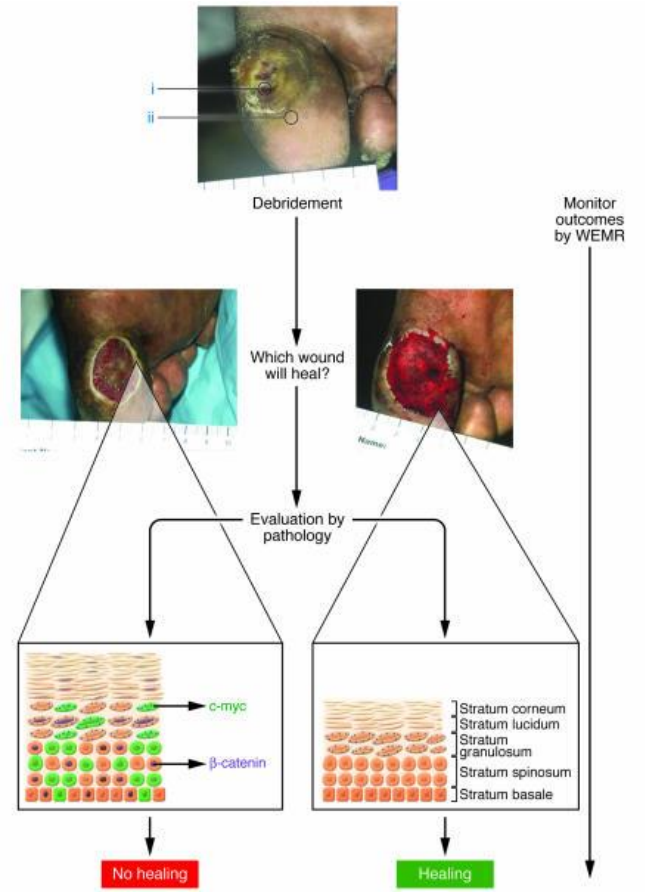

Figure 1: Typical foot ulcer in an individual with diabetes is presented at the top. The no healing area (callus) showing ulcer area with biomolecular markers suggestive of healing weakening. The healing anatomic standard but physiologically reduced cells, which can be motivated to heal [7].

Hyperglycaemia

High blood sugar levels leading to the glycation of collagen (non-enzymatic one) and further proteins and production of what scientifically called advanced glycation end products (AGE). This complex diminishes the liquefying of the extracellular ground and maintains the inflammatory modifications detected in diabetes. A chemotactic element is existing on the diabetic wound place that permits for active employment of the chemotactic reaction

imperfect neutrophil [8]. Many factors may stimulate the development leading to raised IL-8 levels and heightened neutrophil enrolment to diabetic wound. Some potential events were responsible for augmented levels of IL-8 associated with the formation of AGE. It has been documented to motivate pro-inflammatory chemokines formation in endothelium by stimulation of MAPK signalling [9].

Prolonged Inflammation

Diabetes mellitus is categorised via a continuing inflammatory situation and through a sustained inflammatory reaction after the damage that is harmful to injured healing. peoples with diabetes regardless of the type of risk for increasing foot ulcers were documented to show augmented inflammatory cells in the wound tissue and adjacent vessels. Moreover, macrophages kind 1 (M1) to macrophages kind 2 (M2) macrophage percentage, a pointer of chronic inflammation [10] Other studies have presented that the sustained inflammatory phase is considered by uninterrupted expression and augmented levels of pro-inflammatory cytokines, identical interleukin-6 (IL-6) and tumour necrosis factor-a (TNF-a) [11]

Impaired Angiogenesis

Angiogenesis has been labelled to stay weakened in the not fully healing diabetic wound. Within the wound, neovascularization happens as a local factor that motivates nearby cells (angiogenesis) and since of engaged circulating endothelial progenitor cells (EPC) that participate to present and novel vascular networks (angiogenesis). Vascular preservation, healing, and wound remedial cytological and molecular programme in the site of both the bone marrow and the marginal wound are bargained via high blood because of EPC deficiencies [12]. The word “homing” tells to the indications that fascinate and motivate the cells intricate in curative to travel to locations of wound and aid in healing. EPC employment to the injured place correlated to ischemia-stimulating control of stromal cell-derived factor–1α (SDF-1α)[13]. The diminished manifestation of SDF-1α via cutaneous cells such as epithelial cells and myofibroblast might be liable for the absence of EPC homing to the margin of diabetic injuries. This highlights the complication of controlling reactions in diabetic wounds and clarifies the unpredictable reaction to presently accepted hyperoxia procedures in peoples suffering from diabetes [7]

Hypoxia

Abnormal wound healing still a major health question for an enormous number of patients in the world. The physiologic reaction to limited wound hypoxia shows a serious role informative the achievement of the strong or normal curative method. Hypoxia-inducible factor-1 (HIF-1), as the main controller of O_2 homeostasis, is a significant element of healing consequences. HIF-1 participated in all phases of wound repair via its action in cell

relocation, cell persistence under hypoxic environments, cell multiplication, growth factor production, and matrix formulations during the healing development [14].

Microvascular and Macro Vascular Dysfunction

Dysfunctions of Peripheral arterial weakens wound remedial via disturbing major circulation and blood to the inferior extremities. In addition, Neurol pathy has been detected to be related to variations in the minor circulation. O_2 permeation, calculated by O_2 transport and O_2 removal, is reduced in the foot zone of diabetic patients with the presence of neural pathy in comparison with those absent neural pathy as recoded in therapeutic hyper-spectral imaging. This weakening in O_2 infusion is also detected even with the prompt of endothelium vasodilatation by acetylcholine. Moreover, diabetic neuropathy is linked with impairment of vasodilation regardless of dependency and therefore, diminished microcirculation, unrelated to the existence of major vascular disease [15].

Impaired Neurol peptide Signalling

Sensitive nerve fibres of the epidermal area are motivated straightaway next damage and produce numerous Neurol peptides side the situation of the damaged and sections. Neuropeptides as material P, neuropeptide Y and calcitonin gene-related peptide (CGRP) stimulus all cells that are intricate in vascularity regulation and angiogenesis [16]. The manifestation of this neuropeptide has been documented to be diminished in diabetes. In addition, their changed activations and role have directed to the decreased healing detected in diabetic injuries. The axon of neurons -link vasodilation according to (Lewis triple flare response) is not existing. The motivation of the fibres should form backward motivation of neighbouring fibres to produce the substance, including catecholamines and histamine that induce relaxation of blood vessels and Hyperemia throughout wound healing. The weakened Lewis triple-flare response detected in diabetic peoples, consequences ischemic tissues, regardless of the lack of interference macrocirculation [17].

Debridement

It participates in eliminating callus or nodule, necrotic tissue, foreign fragments, and microorganisms and toxins from a wound matrix; all these features prevent injuries remedial. Callus only is recognised to show as an extraneous substance and rise local pressure; eliminating the callus and endorsing the secretion of growth elements transform a prolonged healing situation to serious healing. This aids the wound well capable to react to interesting therapy and eventually heal. There are numerous techniques of debridement, including surgery, enzyme arrangements, polysaccharide drops, and hydrogels. Callus elimination can decrease pressure by 30%, [18]. However, numerous prospective measured research has found that surgical involvements might have a merely partial further rate in ulcer healing associated with conventional therapy.

Revascularization

Arterial disease is unique of simultaneous illnesses in people suffering from diabetes and the indicators of increasing delay repair foot ulcers by amplified risk for essential subtraction. A principle in the therapy of foot ulcer is revascularization. Numerous surgical techniques can be employed, including (angioplasty, endarterectomy, grafting), which is made and the choice of the suitable one is centred on several parameters, including the overall circumstance of the patient, the harshness of the ischemia. In the incident of present contamination, should progress both infection regulator and revascularization in equivalent [19].

Histopathological alteration

Foot ulceration associated with diabetes has intricate and many factors that can include modifications in the pathophysiology of the superficial tissue. The diabetic soft tissue characterised by denser skin, including the epidermis and dermis layers,), fewer interdigitating between these layers, denser elastic septa and diminished adipose cell dimension. histological calculation of the subcutaneous shown a difference rise in the zone segment of elastic borders in healthy tissue against non-diabetic tissue, with an equivalent diminution in the zone section of fat tissue. Certainly, the depths of the border wall width confirmed that the elastic septal were massively thicker in the diabetic tissue [20]. These developments were accompanied by illuminations of the separation of the elastin fibres inside the septal barriers of the diabetic tissue (Figure2). Besides, the septal barriers of the diabetic

skin enclosed collagen fibres that were denser in segments (Table 1) and (Figure3). Degeneration of the fat cells was not detected in some of these samples, although, the adipocytes in diabetic skin seemed to be less even in shape [21]. A consideration of the histomorphological alterations in the diabetic tissue can be worth fully comprehending the biomechanical modifications, which happen in diabetes and the succeeding rise in accountability to collapse.

Table1: Histological analysing by Diabetes Status (means ± SD)

	Diabetic	**Non-Diabetic**	***p***
Skin Thickness [Arithmetic, Harmonic] (µm)	2056 ± 662 [1614 ± 520, 1722 ± 553]	1815 ± 668 [1425 ± 524, 1530 ± 542]	0.4
Dermis Thickness [Arithmetic, Harmonic] (µm)	1103 ± 255 [866 ± 200, 872 ± 198]	688 ± 321 [540 ± 252, 529 ± 271]	0.011
Epidermis Thickness [Arithmetic, Harmonic] (µm)	953 ± 486 [748 ± 381, 797 ± 418]	1127 ± 611 [885 ± 480, 935 ± 499]	0.5
Interdigitating Index	1.90 ± 0.35	2.09 ± 0.30	0.3
Area Fraction of Elastic Septal [Adipose] (%)	67 ± 7 [33 ± 7]	46 ± 16 [54 ± 15]	0.0035
Elastic septal Thickness [Arithmetic, Harmonic] (µm)	270 ± 61 [212 ± 48, 96 ± 31]	151 ± 56 [120 ± 43, 62 ± 20]	0.0003
Adipose Area (µm2)	2160 ± 451	1812 ± 492	0.2
Minimum Adipocyte Diameter (µm)	45.0 ± 4.9	40.7 ± 5.6	0.13

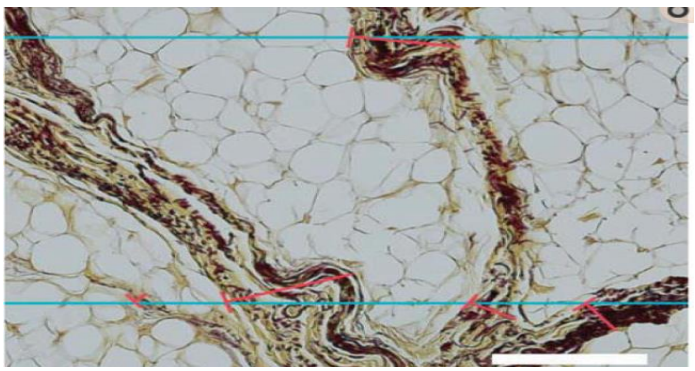

Figure 2: Image shows the dimension of elastic barrier depth in a segment marked with reformed Hart's. Blue outlines refer to the parallel outlines of the analysis; the red lines signify the widths preliminary at the capture of the probe and the left side of the line barriers. Scale bar signifies 200 μm. Adipocytes that were impaired or excessively inaccurate due to treating were not involved in the measurements [21].

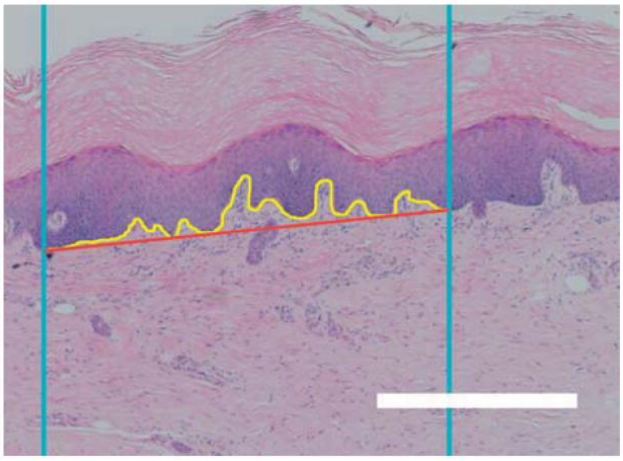

Figure 3: Interdigitating key. Blue outlines signify the lines of the analysis; the red mark signifies the space between the capture of the probe and the skin edge. the yellowish stain signifies the length of the edge, Scale bar 500 μm [21].

Managing of Wound Contamination

Entirely people with diabetes suffering from a foot ulcer, importantly required to evaluated for the occurrence of contamination. Most often diabetic ulcer contamination is recognised by the clinical signs of inflammation, not in experiments results. Nevertheless, the agreement principle aimed at identifying osteomyelitis is an optimistic cell culture from the bone segment; the degree of the contamination should be calculated after the removal of nodules or hard skin and necrotic tissue. Management with antimicrobial is non-essential continuously and medially uncontaminated wounds did not need antibiotics remedy. However, furthermost patients in term of mild-moderate infections can be used consumed antibiotics that are extremely active; in comparison, patients with general inflammatory outcomes should be

estimated for necrotizing contaminations, gangrene, or deep abscesses that regularly need serious surgical interference. In many cases, quick surgical involvement can be precise beneficial as it may diminish the risk of inferior extreme amputation.

Conclusion

Wound healthy closing is a multifaceted natural procedure that includes a great numeral of cell kinds, GF and inflammatory substrate. Diabetes leading reason for weakened wound remedial by disturbing particular or more many biological pathways of the development. Well, considerate of these pathways will tolerate targeted therapy for DFUs. Healing methodologies so far contain critical wound callus, passable force off-loading and local plasters. However, exactly ulcers are extremely resistant to mutual tactics and the perfection of wound curative remains to be the objective of numerous therapy approaches. Stem cell established therapy, gene managements and neuropeptides direction signify a promising therapeutic method for chronic wounds inflexible to typical therapy.

Reference

1. Gurtner GC, Werner S, Barrandon Y, Longaker MT. Wound repair and regeneration. Nature. 2008;453(7193):314–21.
2. Yamaguchi Y, Yoshikawa K. Cutaneous wound healing: an update. J Dermatol. 2001;28(10):521–34.
3. Eckes B, Zigrino P, Kessler D, Holtkötter O, Shephard P, Mauch C, et al. Fibroblast-matrix interactions in wound healing and fibrosis. Matrix Biol. 2000;19(4):325–32.
4. Nayak BS, Sandiford S, Maxwell A. Evaluation of the wound-healing activity of ethanolic extract of Morinda citrifolia L. leaf. Evidence-based Complement Altern Med. 2009;6(3):351–6.
5. Shah JMY, Omar E, Pai DR, Sood S. Cellular events and biomarkers of wound healing. Indian J Plast Surg Off Publ Assoc Plast Surg India. 2012;45(2):220.
6. Brem H, Sheehan P, Rosenberg HJ, Schneider JS, Boulton AJM. Evidence-based protocol for diabetic foot ulcers. Plast Reconstr Surg. 2006;117(7S):193S-209S.
7. Brem H, Tomic-Canic M. Cellular and molecular basis of wound healing in diabetes. J

Clin Invest. 2007;117(5):1219–22.

8. Liu J, Zhao S, Tang J, Li Z, Zhong T, Liu Y, et al. Advanced glycation end products and lipopolysaccharide synergistically stimulate proinflammatory cytokine/chemokine production in endothelial cells via activation of both mitogen-activated protein kinases and nuclear factor-κB. FEBS J. 2009;276(16):4598–606.
9. Lan C-CE, Wu C-S, Huang S-M, Wu I-H, Chen G-S. High-glucose environment enhanced oxidative stress and increased interleukin-8 secretion from keratinocytes: new insights into impaired diabetic wound healing. Diabetes. 2013;62(7):2530–8.
10. Diegelmann RF, Evans MC. Wound healing: an overview of acute, fibrotic and delayed healing. Front biosci. 2004;9(1):283–9.
11. Buckley CD. Why does chronic inflammation persist: An unexpected role for fibroblasts. Immunol Lett. 2011;138(1):12–4.
12. Asahara T, Takahashi T, Masuda H, Kalka C, Chen D, Iwaguro H, et al. VEGF contributes to postnatal neovascularization by mobilizing bone marrow-derived endothelial progenitor cells. EMBO J. 1999;18(14):3964–72.
13. Gallagher KA, Liu Z-J, Xiao M, Chen H, Goldstein LJ, Buerk DG, et al. Diabetic impairments in NO-mediated endothelial progenitor cell mobilization and homing are reversed by hyperoxia and SDF-1α. J Clin Invest. 2007;117(5):1249–59.
14. Hong WX, Hu MS, Esquivel M, Liang GY, Rennert RC, McArdle A, et al. The role of hypoxia-inducible factor in wound healing. Adv wound care. 2014;3(5):390–9.
15. Khazaei M, Moien-Afshari F, Laher I. Vascular endothelial function in health and diseases. Pathophysiology. 2008;15(1):49–67.
16. Theocharidis G, Veves A. Autonomic nerve dysfunction and impaired diabetic wound healing: The role of neuropeptides. Auton Neurosci. 2020;223:102610.
17. Parkhouse N, Le Quesne PM. Impaired neurogenic vascular response in patients with diabetes and neuropathic foot lesions. N Engl J Med. 1988;318(20):1306–9.
18. Edwards J, Stapley S. Debridement of diabetic foot ulcers. Cochrane Database Syst Rev. 2010;(1).
19. Roffi M, Angiolillo DJ, Kappetein AP. Current concepts on coronary revascularization in diabetic patients. Eur Heart J. 2011;32(22):2748–57.
20. Fărcaş-Berechet C-M, Berechet E-M, Crăiţoiu Ş, Mehedinţi M-C, Osman A, Eremia I-A, et al. Clinical, statistical, histological and immunohistochemical aspects of periodontal changes in patients with diabetes mellitus. Rom J Morphol Embryol Rev Roum Morphol Embryol. 2019;60(4):1191–8.
21. Wang Y-N, Lee K, Ledoux WR. Histomorphological evaluation of diabetic and non-diabetic plantar soft tissue. Foot ankle Int. 2011;32(8):802–10.