Assessment of the histological state of the healing wound

Akriti Gupta[1], Pramod Kumar[2]

[1]*Department of Pathology, Seth GS Medical College and KEM Hospital, Mumbai 400012, Maharashtra, India.*
[2]*Department of Plastic Surgery, King Abdulaziz Specialist Hospital, Sakaka 42421, Al-Jouf, Saudi Arabia.*

Address for correspondence: Dr. Akriti Gupta, Department of Pathology, Seth GS Medical College and KEM Hospital, Mumbai 400012, Maharashtra, India. E-mail: dr.akriti@gmail.com

ABSTRACT

The dynamic process of wound healing has various phases, and the knowledge of which is essential for identification of the pathology involved in a chronic intractable wound. Various instruments for the assessment of wound healing have been described, primarily for clinical assessment of the wound. However, very few instruments are currently available for histological grading of the wound. The aim of this article is to review all available literature from 1993 to 2014 on the objective histological scoring of the state of wound healing. This review article emphasizes the importance of histological grading of wounds based on the different parameters from each phase of wound healing and the need for an ideal grading system in order to help assessment of wound status. The parameter chosen in an experimental model should be defined by the scientific question, the underlying hypothesis and the pathogenesis of the disease.

Key words:

Experimental wound assessment, grading of wound, histopathologic grading, wound assessment, wound grading, wound healing, wound histology

INTRODUCTION

The dynamics of wound healing are complex. A thorough understanding of the normal healing process is a prerequisite for unveiling the pathology. Wound healing begins with homeostasis at the site of injury, progresses to an inflammatory phase followed by proliferation of the epithelial and matrix components, and ends with the formation of scar tissue marked by laying down of a highly organized collagen matrix.[1] Various factors, extrinsic and intrinsic to the injured tissue, affect the healing process.[2] These are broadly categorized into local and systemic factors. Factors directly influencing the immediate wound environment are considered to be local factors, while the overall health of the individual affecting his ability to heal constitutes the systemic factors[3] [Table 1].

Access this article online	
Quick Response Code:	Website: www.parjournal.net
	DOI: 10.4103/2347-9264.158862

Impaired wound healing is not an uncommon occurrence in clinical practice. Both local and systemic factors are responsible for impaired healing and weak scar tissue formation.[2] Acute wounds heal following the normal sequence of the healing process. Acute wounds that fail to progress in a timely and orderly fashion through the normal stages of healing are described as chronic wounds.[1] Because of associated early and late complications, chronic wounds remain an intractable clinical problem and a frequent cause of morbidity and mortality.[1]

Various interventions are available for amelioration of impaired healing. Hence, it is important to evaluate wound healing in order to compare the efficacy of

How to cite this article: Gupta A, Kumar P. Assessment of the histological state of the healing wound. Plast Aesthet Res 2015;2:239-42.
Received: *29-12-2014*; **Accepted:** *18-03-2015*

Table 1: Factors influencing the wound healing

Local factors	Systemic factors
Oxygenation	Age, gender
Foreign body	Disease: diabetes, keloids, fibrosis, jaundice, uremia
Blood supply	Medications: NSAIDs, glucocorticoids, chemotherapy
	Stress, nutrition, alcoholism
	Immunocompromised status, AIDS, cancer, radiation

NSAIDs: Nonsteroidal anti-inflammatory medications, AIDS: Acquired immune deficiency syndrome

different interventions. Wound healing is evaluated by both clinical features and biochemical and histological parameters. Nuclear medicine can assist in assessing the vascularity of healing tissue, and hence plays a role in recording inflammation. However, study of the histological features appears to be more reliable as the findings can be recorded photographically for evaluation by different experts.

A literature search was performed on histological scoring of wound from 1993 to 2014. A total of 30 available relevant literatures on wound healing and histological scoring based on various parameters from different stages of wound healing were selected for review.

NORMAL PROCESS OF WOUND HEALING

The sequence of events in normal wound healing has been widely studied and described in literature.[4] Wound healing is a complex biological process that takes place in all tissues in all organs of the body. Various cell types, including keratinocytes, neutrophils, macrophages, lymphocytes, fibroblasts and endothelial cells, are involved in this process.[3] The necrotic tissue is either removed by scavenger cells or separated from living tissue by the process of phagocytosis.

The wound healing process consists of four phases: hemostasis, inflammation, proliferation and remodeling.[1]

Coagulation and hemostasis

The initial step assists in the protection of the vascular system to maintain the functionality of the organ. The clot formed as a result of coagulation provides a matrix for the cells involved in subsequent steps of hemostasis and inflammation.[1] Various pro-inflammatory cytokines and growth factors are released by the clot and wound tissue. Inflammatory cells then migrate to the wound site by the process of chemotaxis and promote the inflammatory phase.[4-6]

Inflammation

The goal of the inflammatory phase is to fight potential bacterial contamination of the wound and to activate cytokine secretion.[1,7] Uncontrolled inflammation can destroy the early migratory effect, leading to an arrest of the healing process.[8]

Proliferation

The proliferation phase overlaps with the preceding inflammatory phase. It represents a proliferation of both epithelial and dermal elements which results in reepithelialization of the wound and laying down of the primary extracellular matrix.[3] Epidermal stem cells and bone marrow derived stem cells also play a role during this phase. Angiogenesis occurs secondary to endothelial progenitor cells, a derivative of hematopoietic stem cells.[9,10]

Wound remodeling leading to scar formation

This phase marks the final step in tissue remodeling and differentiation leading to recovery of the skin and its aesthetic restoration.[8,11] Reconstruction of the dermis occurs by reorganization of the matrix collagen.[7] Fibroblasts differentiation into myofibroblasts, leading to wound contraction and closure.[12]

ASSESSMENT OF THE WOUND

Impaired wound healing occurs secondary to disordered collagen formation[13] and underlying predisposing conditions.[14] In order to effectively manage chronic wounds, periodic assessment of the healing process is necessary.[15] The insights gained from this type of assessment are expected to facilitate the development of novel therapies by stratifying their specific contributions to the wound healing process in time and stage-specific manner.[7] Hence, a standardized and reproducible model is required to obtain information about the wound healing process as well as to better understand the pathology and improve medical technologies.[16] Instruments to assess wound healing can help to enhance communication among clinicians by defining a common language and standardizing assessment of wound characteristics.[15]

Because healing is a dynamic process, it is difficult to evaluate and requires consistent measurements.[17] A complete assessment of the wound must include the size, associated attributes, host factors and environmental factors, all of which impact optimal wound management.[17] In addition, demographics and quality of care also provided aid in assessing the repair process.[17]

Various tools for assessing wound healing clinically have been described, including the Pressure Ulcer Score for Healing (PUSH), the Sussman Wound Healing Tool (SWHT), the Wound Healing Scale, the Leg Ulcer Measurement Tool (LUMT) and the granulometer.[18] However, these instruments can only measure changes in wound healing and do not predict healing or measure wound characteristics.[18] Additional tools to assess the status of the healing wound include Laser-Doppler Flowmetry (LDF) to evaluate cutaneous blow flow and planimetry.[19]

The assessment of the histological state of the healing wound is important in clinical practice for postoperative patient management.[20] Histological evaluation should include the basic components of the healing process including angiogenesis, inflammation, fibroplasia and restoration of the connective tissue matrix, wound contraction and remodeling, epithelialization and differentiation.[17]

Comparison of histologic patterns with the known physiologic variation in tissue morphology assists in

qualitative derivation of the diagnosis. The degree of changes observed when scored on an ordinal scale, namely, low, medium or high grade, provides a semi-quantitative score. On the other hand, the exact quantitative measurement in terms of the absolute number of cells and areas of tissue gives a quantitative score.[21] A quantitative scoring system, while being highly specific and standardized, is difficult to score because in most cases it is difficult to objectify the exact interval between two values.[21] Hence, semi-quantitative scoring systems remain in wide use in the world of the biomedical research.

Various studies have been conducted, and wound healing models have been proposed to understand the normal healing process and to standardize the semi-quantitative and quantitative evaluation of selected parameters of wound healing. In a study assessing wound healing in the maxillofacial region, Sultana *et al.*[20] utilized scoring of 6 histological parameters to give a healing score [Table 2]. The total healing score in each case was calculated by adding the scores of individual criteria, with lower scores indicating poorer wound healing. Healing status was graded as good (16-19), fair (12-15) and poor (8-11). Using this healing score, Sultana *et al.*[20] concluded that risk factors in the study group were correlated with delayed wound healing in comparison to the control group.

While studying the overall process of wound healing, Braiman-Wiksman *et al.*[7] evaluated the role of multiple processes involving the skin components including the epidermis, dermis, hypodermic, blood vessel and connective tissue [Table 3]. They stressed an objective assessment and quantification of wound healing. Using a quantitative assessment method, the authors provide insight into the specific defects found at various stages, which involve a variety of cells and pathways in the process of wound healing.

In their experimental model of open-skin wound healing in corticosteroid-treated and diabetic rats, Gal *et al.*[22] used both semi-quantitative and quantitative methods in a time- and stage-bound assessment of wound healing [Table 4]. Consistent with previous studies,[22,23] they concluded that there is only a quantitative difference between primary and secondary wound healing. In contrast to the quantitative method, the semi-quantitative scoring system can evaluate keratinization, suggesting that keratinocyte differentiation is important in wound healing. Hence, a quantitative assessment alone is not sufficient to demonstrate significant differences in skin wound healing.

Lemo *et al.*[21] provided a mathematical model for healing and a remodeling index in experimental skin wounds. The mathematical model involves measurement of five specific parameters [Table 5], based on which three indices can be determined: the superficial contraction index (SCI), the deep contraction index (DCI) and the wound contraction index (WCI). These indices, however, measure only the contraction of the wound, which represents the initial stage of healing. To assess the mid- and long-term healing process, Lemo *et al.*[21] provide the global healing index (GHI), given by the formula GHI = SCI + DCI − WCI. This index allows scoring of the healing process and follow-up of its progress.

Tascilar *et al.*[24] used Abramov's histologic scoring system to demonstrate the effectiveness of N-acetyl cysteine administration in alleviation of the negative effects of radiotherapy on incisional wound healing. Abramov's histologic scoring system encompasses a semi-quantitative

Table 2: Parameters assessed to calculate healing score

Number	Histological Parameter
1	Amount of granulation tissue (profound-1, moderate-2, scanty-3, absent-4)
2	Inflammatory infiltrate (plenty-1, moderate-2, a few-3)
3	Collagen fiber orientation (vertical-1, mixed-2, horizontal-3)
4	Pattern of collagen (reticular-1, mixed-2, fascicle-3)
5	Amount of early collagen (profound-1, moderate-2, minimal-3, absent-4)
6	Amount of mature collagen (profound-1, moderate-2, minimal-3)

Number 1-4: H and E, Number 5-6: Masson's trichrome stain, old collagen fibers take deep blue color and the new collagen fibers stain light blue

Table 3: Histological skin cell parameters for the assessment of wound healing

Healing parameter	Assessment parameter
Epidermal closure	Basal layer of the epidermis to assess the newly formed epidermis
Epidermal differentiation	Spinous epidermal differentiation (early) Granular epidermal differentiation (late)
Epidermal migration	Migrating cells
Granulation tissue formation and Epidermal hyperplasia	Proliferating cells
Granulation tissue and matrix formation	Collagen fiber deposition
Inflammation dermal closure	White blood cells abscesses matrix remodeling
Late stage of matrix remodeling	Elastin fiber deposition

Table 4: Parameters of histologic assessment of wound

Semi-quantitative method	Quantitative method
Wound reepithelialization: migration of keratinocytes, bridging of cells, keratinization	Polymorphonuclear leucocytes/ tissue macrophages ratio
Inflammatory cells: absence/ presence (mild/moderate/marked)	Percentage of reepithelialization
Fibroblasts: absence/presence (mild/moderate/marked)	Area of the granulation tissue
New vessels: absence/presence (mild/moderate/marked)	-
Collagen: absence/presence (mild/moderate/marked)	-

Table 5: Parameters measured in the mathematical model

Length of the reepithelialization zone (L)
Distance between the borders of the wound (S)
Depth of the wound (D)
Thickness of the connective tissue (T)
Thickness of the natural dermis on both sides of the wound (N)

scoring of acute and chronic inflammation, the amount of granulation tissue, the level of fibroblast maturation, the amount of collagen deposition and the level of reepithelialization and neovascularization.[25]

Ancillary techniques such as special stains and immunohistochemistry in addition to light microscopic examination can help in the accurate assessment of the components of a healing wound. For instance, Masson's trichrome staining is used to demonstrate the presence of collagen in the healing wound.[26,27] In addition, various immunohistochemical markers have been used to demonstrate the components of the healing wound, such as antiloricrin for epithelial differentiation,[26] CD31 for angiogenesis[28] and antibodies against cytokine ligands and receptors.[29] Some authors have studied apoptosis using Annexin V-FITC binding assay[30] and TUNEL Assay.[26]

Histopathology has always been the gold standard in diagnosing certain infectious, degenerative or neoplastic diseases in humans and animals.[21] The number of studies performed to provide a standardized system for histological evaluation of the wound demonstrates the importance of histopathology. Careful assessment of chronic wounds can shed light on the exact pathology and assist in developing a strategy for further management. It can also be a powerful tool in the evaluation of the effect of novel drugs on wound healing.[19] Histopathology also provides information on the usefulness of combination therapy and determining effective drug dosage in order to minimize adverse effects.

There are numerous scoring systems provided by various pioneers in the field. However, the need for uniformity persists. Although the selection of parameters in most scoring systems is generally based on a basic knowledge of the wound healing, the parameters chosen in an experimental model should be defined by the scientific question, the underlying hypothesis and the pathogenesis of the disease.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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. Kumar V, Abbas AK, Fausto N, Aster JC. Inflammation and repair. Robbins and Cotran Pathologic Basis of Disease. 9th ed. Philadelphia: Elsevier Saunders; 2014. p. 69-110.
3. Guo S, Dipietro LA. Factors affecting wound healing. *J Dent Res* 2010;89:219-29.
4. Gosain A, DiPietro LA. Aging and wound healing. *World J Surg* 2004;28:321-6.
5. Broughton G 2nd, Janis JE, Attinger CE. The basic science of wound healing. *Plast Reconstr Surg* 2006;117:S12-34.
6. Campos AC, Groth AK, Branco AB. Assessment and nutritional aspects of wound healing. *Curr Opin Clin Nutr Metab Care* 2008;11:281-8.
7. Braiman-Wiksman L, Solomonik I, Spira R, Tennenbaum T. Novel insights into wound healing sequence of events. *Toxicol Pathol* 2007;35:767-79.
8. Diegelmann RF, Evans MC. Wound healing: an overview of acute, fibrotic and delayed healing. *Front Biosci* 2004;9:283-9.
9. Wu Y, Wang J, Scott PG, Tredget EE. Bone marrow-derived stem cells in wound healing: a review. *Wound Repair Regen* 2007;15 Suppl 1:S18-26.
10. Rea S, Giles NL, Webb S, Adcroft KF, Evill LM, Strickland DH, Wood FM, Fear MW. Bone marrow-derived cells in the healing burn wound-more than just inflammation. *Burns* 2009;35:356-64.
11. Hackam DJ, Ford HR. Cellular, biochemical, and clinical aspects of wound healing. *Surg Infect (Larchmt)* 2002;3 Suppl 1:S23-35.
12. Gabbiani G. The myofibroblast in wound healing and fibrocontractive diseases. *J Pathol* 2003;200:500-3.
13. Baum CL, Arpey CJ. Normal cutaneous wound healing: clinical correlation with cellular and molecular events. *Dermatol Surg* 2005;31:674-86.
14. Gohel MS, Taylor M, Earnshaw JJ, Heather BP, Poskitt KR, Whyman MR. Risk factors for delayed healing and recurrence of chronic venous leg ulcers-an analysis of 1324 legs. *Eur J Vasc Endovasc Surg* 2005;29:74-7.
15. Mullins M, Thomason SS, Legro M. Monitoring pressure ulcer healing in persons with disabilities. *Rehabil Nurs* 2005;30:92-9.
16. Motlík J, Klíma J, Dvoránková B, Smetana K Jr. Porcine epidermal stem cells as a biomedical model for wound healing and normal/malignant epithelial cell propagation. *Theriogenology* 2007;67:105-11.
17. Lazarus GS, Cooper DM, Knighton DR, Percoraro RE, Rodeheaver G, Robson MC. Definitions and guidelines for assessment of wounds and evaluation of healing. *Wound Repair Regen* 1994;2:165-70.
18. Pillen H, Miller M, Thomas J, Puckridge P, Sandison S, Spark JI. Assessment of wound healing: validity, reliability and sensitivity of available instruments. *Wound Pract Res* 2009;17:208-17.
19. Karayannopoulou M, Tsioli V, Loukopoulos P, Anagnostou TL, Giannakas N, Savvas I, Papazoglou LG, Kaldrymidou E. Evaluation of the effectiveness of an ointment based on Alkannins/Shikonins on second intention wound healing in the dog. *Can J Vet Res* 2011;75:42-8.
20. Sultana J, Molla MR, Kamal M, Shahidullah M, Begum F, Bashar MA. Histological differences in wound healing in maxillofacial region in patients with or without risk factors. *Bangladesh J Pathol* 2009;24:3-8.
21. Lemo N, Marignac G, Reyes-Gomez E, Lilin T, Crosaz O, Ehrenfest DM. Cutaneous reepithelialization and wound contraction after skin biopsies in rabbits: a mathematical model for healing and remodelling index. *Vet Arh* 2010;80:637-52.
22. Gal P, Kilik R, Mokry M, Vidinsky B, Vasilenko T, Mozes S, Lenhardt L. Simple method of open skin wound healing model in corticosteroid-treated and diabetic rats: standardization of semi-quantitative and quantitative histological assessments. *Vet Med* 2008;53:652-9.
23. Barbul A, Regan MC. Biology of wound healing. In: Fischer JA, editor. Surgical Basic Science. St. Louis: Mosby Year-Book; 1993. p. 68-88.
24. Tascilar O, Cakmak G, Emre A, Bakkal H, Kandemir N, Turkcu U, Demir E. N-acetylcycsteine attenuates the deleterious effects of radiation therapy on inci-sional wound healing in rats. *Hippokratia* 2014;18:17-23.
25. Abramov Y, Golden B, Sullivan M, Botros SM, Miller JJ, Alshahrour A, Goldberg RP, Sand PK. Histologic characterization of vaginal *vs.* abdominal surgical wound healing in a rabbit model. *Wound Repair Regen* 2007;15:80-6.
26. Lee YH, Chang JJ, Chien CT, Yang MC, Chien HF. Antioxidant sol-gel improves cutaneous wound healing in streptozotocin-induced diabetic rats. *Exp Diabetes Res* 2012;2012:504693.
27. Piskin A, Altunkaynak BZ, Tümentemur G, Kaplan S, Yazici OB, Hökelek M. The beneficial effects of *Momordica charantia* (bitter gourd) on wound healing of rabbit skin. *J Dermatolog Treat* 2014;25:350-7.
28. Huang SP, Huang CH, Shyu JF, Lee HS, Chen SG, Chan JY, Huang SM. Promotion of wound healing using adipose-derived stem cells in radiation ulcer of a rat model. *J Biomed Sci* 2013;20:51.
29. Zheng Z, Lee KS, Zhang X, Nguyen C, Hsu C, Wang JZ, Rackohn TM, Enjamuri DR, Murphy M, Ting K, Soo C. Fibromodulin-deficiency alters temporospatial expression patterns of transforming growth factor-ß ligands and receptors during adult mouse skin wound healing. *PLoS One* 2014;9:e90817.
30. Kim SW, Zhang HZ, Guo L, Kim JM, Kim MH. Amniotic mesenchymal stem cells enhance wound healing in diabetic NOD/SCID mice through high angiogenic and engraftment capabilities. *PLoS One* 2012;7:e41105.