

SCIENTIFIC DISCIPLINES, PHYTOTHERAPY AND BIOMARKERS OF WOUND HEALING

¹Reshma Selvarajan, ²Kousalya M, ³Priyanka R

Department of Pharmacology
C.L Baid Metha College of Pharmacy,
Rajiv Gandhi Salai, Thoraipakkam
Chennai-600041, Tamil Nadu.

Correspondence to Author:
Ms Reshma Selvarajan

Abstract- Wound healing is one of the most complex processes in the human body. It involves the spatial and temporal synchronization of a variety of cell types with distinct roles in the phases of hemostasis, inflammation, growth, re-epithelialization, and remodeling. The process of wound healing is a well-coordinated series of events in which a multitude of variables are either stimulated or inhibited. The first stage is an inflammatory shift accompanied by angiogenesis, re-epithelialization and the activation of innate immunity. This is followed by the proliferation phase. Changes in the microenvironment including alterations in mechanical forces, oxygen levels, chemokines, extracellular matrix and growth factor synthesis directly impact cellular recruitment and activation, leading to impaired states of wound healing. Single cell technologies can be used to interpret these cellular alterations in diseased states such as in chronic wounds and hypertrophic scarring so that effective therapeutic solutions for healing wounds can be developed. Identification of numerous potential biomarkers using different avenues of sample collection and molecular approaches is currently underway. A focus on simplicity and consistent implementation of these biomarkers, as well as an emphasis on efficacious follow-up therapeutics, is necessary for transition of this technology to clinically feasible point-of-care applications

Keywords: Wound healing, Inflammation, Medicinal Plants, Biomarkers, Cell proliferation.

1. INTRODUCTION

The four separate and overlapping phases of wound healing—haemostasis, inflammation, proliferation, and remodelling—are often smoothly progressed through by acute wounds in an organized and efficient manner.^{[2][4][45]} The process of wound healing is complex and involves a variety of specialized cells, such as platelets, macrophages, fibroblasts, epithelial and endothelial cells. On the other hand, chronic wounds will similarly initiate the healing process, but will have prolonged inflammatory, proliferative or remodelling phases, which result in tissue fibrosis along with non-healing ulcers.^{[4][45]} Both the extracellular matrix and these cells communicate with one another.^{[2][45]} Proteins and glycoproteins, such as cytokines, chemokines, growth factors, inhibitors and their receptors, also impact healing in addition to the different cellular interactions.^[45]

THE SKIN

The skin is divided into three layers: the epidermis, the dermis, and the sub-cutaneous fat. The epidermis and dermis represent the two most important features of the skin with numerous functions. The subcutaneous fat on the other hand has limited function and makes up the bottom layer of the skin. The epidermis is composed of four cell types. The most common cell is the keratinocyte responsible for making keratin, the skin barrier, photoprotection, home to immune related cells, the Langerhans' cells, and other functions. The epidermis also contains melanocytes, Merkel cells, and lymphocytes. The Langerhans' cells are of great importance in presenting antigens for sensitization to lymphocytes in the skin and in the lymph nodes^[1]. Unmyelinated axons penetrate the skin. Hair follicles extend from the dermis into the subcutaneous fat. The dermis has two basic layers. The thin papillary dermis lies beneath the epidermis and is similar to the connective tissue that invests the appendages. Beneath the papillary dermis is the main portion of the dermis known as the reticular dermis. The superficial vascular plexus lies at the junction of the papillary and reticular dermis and is the site of most cutaneous inflammatory disorders. Vessels, nerves, lymphatics, and the appendages course through the reticular dermis. From the vessels, there is the egress of the lymphocytes and other inflammatory cells that are involved in cutaneous inflammation, infection, immune disorders, and in response to trauma among other conditions.^[9]

1.1 LAYERS OF SKIN

The skin consists of two layers, the epidermis and the dermis. Epidermis is a terminally differentiated stratified squamous epithelium, the major cell type of which is the keratinocyte. Keratinocytes synthesize keratin, a protein containing coiled polypeptide chains which combine to form supercoils of several polypeptides linked by disulphide bonds between adjacent cysteine amino acids. Keratinocytes also produce cytokines in response to injury. ^{[1][2]}

1.1.1. EPIDERMIS

i. Stratum basale (basal cell layer)

This layer is generally only one cell thick, but in glabrous skin and hyperproliferative epidermis it can be two to three cells thick. The main cell type is the keratinocyte that may be dividing or non-dividing. Melanocytes are present in the basal layer and make up 5e10% of the cell population. ^{[1][2]}

ii. Stratum spinosum (spinous or prickle cell layer)

Basal cells move towards the surface and form a layer of polyhedral cells which are connected by desmosomes. These are the 'prickles' seen under the microscope. Within this layer Langerhans cells can be identified. ^{[1][2]}

iii. Stratum granulosum. (Granular cell layer)

Keratinocytes in the granular layer contain intracellular granules of keratohyalin. The cytoplasm also contains smaller lamellated granules (Odland bodies). The cells discharge their lipid components into the intercellular space which plays an important role in barrier function and intercellular cohesion within the stratum corneum. ^{[1][2]}

iv. Stratum corneum (horny layer)

This is the outermost layer of the epidermis. It is comprised of cells that have migrated from the stratum granulosum. The cells (now called corneocytes) have lost their nuclei and cytoplasmic organelles. The cells appear flattened and the keratin filaments align into disulphide cross-linked macrofibrils. This layer may be several cells thick on the palms and the soles, but is less thick elsewhere. In palmoplantar skin there is an additional zone, the stratum lucidum. The cells found in this layer are still nucleated and are termed transitional cells. The time from cell division to shedding from the horny layer is approximately 28 days, but this can be altered in various disease processes. ^{[1][2]}

1.1.2. DERMIS

The dermis is bounded externally by its junction with the epidermis and internally by subcutaneous fat. The dermis is a tough, resilient layer that protects the body against mechanical injury and contains specialized structures. The papillary dermis is the thin upper layer of the dermis. ^{[2][3]} Deeper to this is the reticular dermis also contains cells, ground substance and fibres. The ground substance consists of polysaccharides and proteins which interact to produce hygroscopic proteoglycan macromolecules. ^[2] The cells are fibroblasts that synthesize collagen and elastin fibres. Collagen represents 75% of the dry weight and up to 30% of the volume of the dermis. Seventy-five percent is type I collagen and 15% type III collagen. ^[3] The properties of collagen change both qualitatively and quantitatively with ageing. Elastin fibres are also present within the dermis and these provide a degree of elasticity to the skin. ^{[2][3]}

1.2. TYPES OF WOUNDS:

Wounds can be caused in a number of different ways by a variety of different objects, be it blunt, sharp or projectile. ^[43] They are classified into several categories dependent on the cause and resulting injury: ^{[2][6]}

- i. **Incised wound** – A clean, straight cut caused by a sharp edge (i.e. a knife). Tends to bleed heavily as multiple vessels may be cut directly across. Connecting structures such as ligaments and tendons may also be involved. ^[43]
- ii. **Laceration** – A messy looking wound caused by a tearing or crushing force. Doesn't tend to bleed as much as incised wounds but often causes more damage to surrounding tissues. ^[43]
- iii. **Abrasion** – A wound caused by a scraping force or friction. Tends not to be very deep but can often contain many foreign bodies such as dirt (i.e. after a fall on loose ground). ^[43]
- iv. **Puncture** – A deep wound caused by a sharp, stabbing object (i.e. a nail). May appear small from the outside but may damage deep tissues. Particularly dangerous on the chest, abdomen or head where major organs are at risk. ^[43]
- v. **Avulsion** – A wound caused by a tearing force in which tissue is torn away from its normal position. May bleed profusely depending on the size and location. The tissue is often completely detached. ^[43]
- vi. **Amputation** – The loss of a distinct body part such as a limb, finger, toe or ear. Often very severe with profuse bleeding. In the cases of limb loss this is a medical emergency. ^[43]

1.3. PHASES OF WOUND HEALING:

The complicated mechanism of wound healing occurs in four phases: hemostasis, inflammation, proliferation, and remodelling. ^{[6][7]}

i. Hemostasis

Hemostasis is the first stage in wound healing that can last for two days. As soon as there is a wound on the body, the blood vessels in the wound area constrict to reduce the blood flow.^{[6][7]} This is known as vasoconstriction. At the same time, clotting factors are released at the wound site to coagulate with fibrin, resulting in a thrombus, which is more commonly known as a blood clot. The clot acts as a seal between the broken blood vessels to prevent blood loss.^[6]

ii. Inflammation

The second phase of wound healing is called the Inflammatory Phase. It involves phagocytic cells that release reactive oxygen species, lasting for up to seven days in acute wounds and longer in chronic wounds.^[6] During this phase, white blood cells and some enzymes enter the wound area to stave off infection by clearing bacteria and debris and preparing the wound bed for new tissue growth. Physical characteristics of the phase include inflammation or redness at the wound site, edema, heat, and pain.^[7]

iii. Proliferation

Phase three of wound healing, the Proliferative Phase, focuses on filling and covering the wound. As inflammatory cells undergo apoptosis, wound healing progresses to the proliferation phase, which is characterized by the formation of granulation tissue, angiogenesis (blood vessel formation), wound contraction, and the process of epithelialization.^[6] The new tissue is generally red or pink in appearance due to the presence of inflammatory agents. The time it takes for tissue regeneration depends on the production of collagen proteins by fibroblasts, which is a type of cell found in the connective tissue. This phase of wound healing can last for four days to up to three weeks or more.^{[6][7]}

iv. Remodeling

Scar tissue formation characterizes the final Remodeling Phase (also known as Maturation). It may occur over months or years, depending on the initial severity of the wound, its location, and treatment methods.^[6] During this phase, the new tissue gradually becomes stronger and more flexible. Collagen production continues to build the tensile strength and elasticity of the skin. The build-up of collagen in the granulation tissue leads to scar tissue formation, which is 20 percent weaker and less elastic than pre-injured skin.^{[6][7]}

2. ETIOLOGY:

There are several factors that may impair the wound healing process, including: the pre-existing integrity of the wounded skin due to age or medical treatments, comorbidities, medications, infection, hydration state, nutritional status, lifestyle habits, and pre- and post-operative care if surgery has occurred. Relevant comorbidities, medications, and lifestyle factors include:^[9]

- **Diabetes:** A common complication associated with diabetes is peripheral neuropathy leading to foot ulceration.^{[9][13]} An additional complication is peripheral ischemia secondary to peripheral artery disease. Both complications affect the proliferative phase of healing and lead to the overall slowing of wound healing.
- **Obesity:** Obesity is associated with an increased risk of ischemia and inadequate tissue oxygenation, which may lead to slowed wound healing or necrosis.^{[9][13]}
- **Necrosis:** Unplanned tissue death is another factor that may impede wound healing, requiring debridement to remove the affected tissue surgically before healing can proceed.^[9]
- **Poor nutrition:** Malnutrition (seen frequently in elderly patients), specifically inadequate protein intake, can lead to decreased blood vessel formation, collagen production, and fibroblast proliferation, which ultimately slows the wound healing process.^[13]
- **NSAIDs (non-steroidal anti-inflammatory drugs):** The mechanism of pain reduction by NSAIDs occurs through the inhibition of PGE2, an inflammation mediator. NSAIDs are known to slow wound healing through the halting of angiogenesis. NSAIDs also increase scar formation, particularly if used during the proliferative phase.^[9]
- **Steroids:** The anti-inflammatory and immunosuppressive effects of steroids can hinder wound healing by decreasing fibroblast proliferation and collagen production.^{[13][14]}
- **Radiation therapy:** Ionizing radiation beams can damage epithelial cells as they pass through to targeted tissues, causing skin tissue breakdown and slowed healing of existing and new wounds.^{[13][14]}
- **Chemotherapy:** Chemotherapeutic agents affect wound healing by delaying the inflammatory phase of healing and decreasing collagen production.^{[13][14]}
- **Smoking:** Cigarette smoking, specifically the use of nicotine, affects blood flow by causing vasoconstriction. Nicotine also decreases the body's immune response, which could lead to an increased likelihood of wound infection.^[14]
- **Alcohol:** Alcohol intake is often associated with poor nutritional habits, which may result in decreased immune function. In addition, alcohol may impair wound healing by decreasing angiogenesis and collagen formation, leading to weaker scar tissue formation and an overall slower healing process. Wound healing may also be positively impacted by the addition of certain supplements such as zinc and vitamin C.^{[13][14]}

3. MEDICINAL PLANTS

The use of medicinal plants in treating wounds has been very encouraging, revealing their potential to be used for the preparation of new drugs.^[38] Ethnobotany and ethnopharmacological studies identified the treatment methods used in various cultures and regions. Recently, these effects have been confirmed by various preclinical and clinical studies.^{[38][39]} These studies were mostly conducted to evaluate the effects of local use of traditional extracts such as boiled or herbal powder on burn injuries. However, severe burn injury often results in complications and complex metabolic changes.^[38] These complications are usually associated with oxidative stress. Therefore, topical and systemic administration of medicinal plants, which mostly have antioxidant activities, should be considered in these patients.^{[38][39]} The obtained results indicated that 139 effective medicinal plants were used for wound healing, obtained from ethnobotanical sources of Iran. *Salvia officinalis*, *Echium amoenum*, *Verbascum*, *Glycyrrhiza glabra*, *Medicago sativa*, *Mentha pulegium*, *Datura stramonium* L., *Alhagi* spp., *Aloe vera*, *Hypericum perforatum*, and *Pistacia atlantica*, *Prosopis cineraria* were the most important and effective medicinal plants. Study and identification of effective medicinal plants can give us insight into the preparation of effective medicinal products.^[38] This research aimed to review the constituents of medicinal plants that are beneficial for wound healing.^{[38][39]} A wound is often associated with an increase in oxidative stress and this increase in ROS or other reactive species results in damaging cytotoxicity and delay in wound healing. Hence, it seems that elimination or reduction of ROS can be a good strategy to improve wound healing.^[33] Nowadays, the strategy to target ROS via antioxidants is being highlighted in chronic wound therapy. Most of these plants and/or their components have antioxidant activities. Hence, it seems these plants accelerate wound healing partially through the amelioration of the redox level. However, each plant may have a specific compound(s) that can contribute its effect.^{[38][39]}

4. BIOMARKERS

- An objectively quantifiable material that may be evaluated as a pharmacological response to a therapeutic intervention or as an indicator of a typical physiological or pathological process is called a biomarker.^[25] Therefore, biomarkers can be classified as suggestive, diagnostic and predictive depending on the kind of information they provide, however they are not mutually exclusive.^{[24][26]} One way to forecast results or estimate the chance of a treatment's success is through predictive biomarkers.^[25] When modifying treatment techniques for particular patient populations, they can be a very effective instrument. A single or a combination of factors that may have an impact on the clinical outcome can be detected using diagnostic biomarkers.^[24] One way to track the course of an illness and/or its response to treatment in real time is by using an indicative biomarker.^[26] Finding compounds that impede or regulate healing is the first step in developing biomarkers that are therapeutically useful. These molecules are then validated in patients. Various high-throughput "omics" techniques that utilise human wounds as a biomaterial source are being utilised to comprehend the mechanisms of damage, hence expediting the identification of plausible biomarkers.^[26] It is anticipated that these biomarkers would enable tailored evaluation, forecast patients' state of recovery and offer perceptions into treatment strategies. Such biomarkers are expected to enable tailored evaluation, offer forecasts regarding patients' prognosis for recovery and offer insights into treatment strategies.^{[26][27][28]}

CURRENT STATUS OF BIOMARKERS IN CHRONIC WOUNDS

1) Tissue Biomarkers

Once molecular markers are identified and verified, tissue specimens are a valuable resource for obtaining information related to diagnosis, therapeutic direction and outcome prediction. Histology has long been recognized as a diagnostic tool for conditions like renal illness, transplant rejection and vasculitis. Its most well-known use is in the detection of cancer and precancerous lesions when combined with various molecular marker.^{[26][27]} Over the last ten years, insights obtained from tissue samples obtained from patients have identified the initial molecular indicators linked to the obstruction of healing. Tissue taken from the periphery of nonhealing wounds exhibits enhanced cellular infiltration, hyperproliferative epidermis and varying degrees of fibrosis. However, this method might be highly helpful for early diagnosis, which would allow for more individualized treatment plans.^[26]

2) Wound Fluid Biomarkers

The MMPs are metalloproteinase enzymes, and their regulators, tissue inhibitor of MMPs (TIMPs), play important role in wound healing. MMPs are necessary for a wound to heal effectively, but only at the right level, in the right place, and for the right amount of time.^{[26][27]} Therefore, the correlation between nonhealing in chronic wounds and persistently high levels of MMPs, decreased levels of TIMPs, or abnormalities in their ratio is not surprising. Research has demonstrated that reducing MMP-2 tissue levels is indicative of wound healing itself, and that therapeutic approaches that lower MMP activity—such as MMP absorbent collagen/oxidized regenerated

cellulose dressings—promote the healing of stalled wounds. These results suggest that MMPs may be useful biomarkers for wound healing that are suggestive, predictive, and diagnostic. ^[26]

CONCLUSION

The potential use of medicinal plants in wound care has proven that they can be utilized to manufacture new medicinal products. Research in the fields of ethnobotany and ethnopharmacology has shown how treatments are employed in numerous cultures and environments. ^[40] Several preclinical and clinical investigations conducted recently have supported these benefits. The primary purpose of these investigations was to evaluate the impact of frequently used traditional extracts, like boiling or herbal powder, on burn injuries. ^{[40][42]} However, complicated metabolic alterations and consequences are frequently the outcome of severe burn injuries. Such problems are generally associated with oxidative stress. Because medicinal plants mostly have antioxidant properties, topical and systemic administration of these plants should be taken into consideration. ^[40]

Better diagnostic tools are of vital importance in the clinical field, but they are taking a while to develop. The drive to identify clinically relevant biomarkers originates in the desire to identify patients who, should SOC therapy fail before receiving treatment, would benefit from early therapeutic interventions catered to their individual prognosis for healing. ^{[40][41][42]} This would ultimately lead to better and more economical outcomes. There are now numerous interesting biomarkers being developed that use various methods of sample collecting. Efficient follow-up therapies and a focus on the straightforward, cost-effective, and consistent application of these biomarkers are essential as we move towards point-of-care for medical purposes and useful technological innovations. ^{[40][41]}

REFERENCES:

1. Sharma A, Zakka LR, Mihm Jr MC. Anatomy of the human skin and wound healing. In *Bio engineering In Wound Healing: A Systems Approach 2017* (pp. 27-57).
2. George Broughton II, Janis JE, Attinger CE. The basic science of wound healing. *Plastic and reconstructive surgery*. 2006 Jun 1;117(7S):12S-34S.
3. Gonzalez AC, Costa TF, Andrade ZD, Medrado AR. Wound healing-A literature review. *Anais brasileiros de dermatologia*. 2016 Sep;91:614-20.
4. Rodriguez PG, Felix FN, Woodley DT, Shim EK. The role of oxygen in wound healing: a review of the literature. *Dermatologic surgery*. 2008 Sep;34(9):1159-69.
5. Sharma S, Rai VK, Narang RK, Markandeywar TS. Collagen-based formulations for wound healing: A literature review. *Life sciences*. 2022 Feb 1;290:120096.
6. Weinstein ML. Update on wound healing: a review of the literature. *Military medicine*. 1998 Sep 1;163(9):620.
7. Murphy PS, Evans GR. Advances in wound healing: a review of current wound healing products. *Plastic surgery international*. 2012;2012.
8. Miron RJ, Fujioka-Kobayashi M, Bishara M, Zhang Y, Hernandez M, Choukroun J. Platelet-rich fibrin and soft tissue wound healing: a systematic review. *Tissue Engineering Part B: Reviews*. 2017 Feb 1;23(1):83-99.
9. Muntean A, Stoica I, Enescu DM. Scarless wound healing—a literature review. *Ro J Pediat*. 2019 Jul 1;68(3):160-5.
10. Han G, Ceilley R. Chronic wound healing: a review of current management and treatments. *Advances in therapy*. 2017 Mar;34:599-610.
11. Almadani YH, Vorstenbosch J, Davison PG, Murphy AM. Wound healing: A comprehensive review. In *Seminars in plastic surgery 2021 Jul 15* (Vol. 35, No. 03, pp. 141-144). 333 Seventh Avenue, 18th Floor, New York, NY 10001, USA: Thieme Medical Publishers, Inc..
12. Cañedo-Dorantes L, Cañedo-Ayala M. Skin acute wound healing: a comprehensive review. *International journal of inflammation*. 2019 Jun 2;2019.
13. Pastar I, Stojadinovic O, Yin NC, Ramirez H, Nusbaum AG, Sawaya A, Patel SB, Khalid L, Isseroff RR, Tomic-Canic M. Epithelialization in wound healing: a comprehensive review. *Advances in wound care*. 2014 Jul 1;3(7):445-64.
14. Sorg H, Tilkorn DJ, Hager S, Hauser J, Mirastschijski U. Skin wound healing: an update on the current knowledge and concepts. *European Surgical Research*. 2017 Dec 15;58(1-2):81-94.
15. Pereira RF, Bartolo PJ. Traditional therapies for skin wound healing. *Advances in wound care*. 2016 May 1;5(5):208-29.
16. Moulin V. Growth factors in skin wound healing. *European journal of cell biology*. 1995 Sep 1;68(1):1-7.
17. Tottoli EM, Dorati R, Genta I, Chiesa E, Pisani S, Conti B. Skin wound healing process and new emerging technologies for skin wound care and regeneration. *Pharmaceutics*. 2020 Aug;12(8):735.

18. Lima CC, Pereira AP, Silva JR, Oliveira LS, Resck MC, Grechi CO, Bernardes MT, Olimpio FM, Santos AM, Incerpi EK, Garcia JA. Ascorbic acid for the healing of skin wounds in rats. *Brazilian Journal of Biology*. 2009;69:1195-201.
19. Enoch S, Leaper DJ. Basic science of wound healing. *Surgery (Oxford)*. 2008 Feb 1;26(2):31-7.
20. Beldon P. Basic science of wound healing. *Surgery (Oxford)*. 2010 Sep 1;28(9):409-12.
21. Ho JK, Hantash BM. The principles of wound healing. *Expert Review of Dermatology*. 2013 Dec 1;8(6):639-58.
22. Solati K, Karimi M, Rafieian-Kopaei M, Abbasi N, Abbaszadeh S, Bahmani M. Phytotherapy for wound healing: The most important herbal plants in wound healing based on iranian ethnobotanical documents. *Mini reviews in medicinal chemistry*. 2021 Mar 1;21(4):500-19.
23. Sharma A, Zakka LR, Mihm Jr MC. Anatomy of the human skin and wound healing. In *Bioengineering In Wound Healing: A Systems Approach 2017* (pp. 27-57).
24. Patel S, Maheshwari A, Chandra A. Biomarkers for wound healing and their evaluation. *Journal of wound care*. 2016 Jan 2;25(1):46-55.
25. Yussof SJ, Omar E, Pai DR, Sood S. Cellular events and biomarkers of wound healing. *Indian Journal of Plastic Surgery*. 2012 May;45(02):220-8.
26. Lindley LE, Stojadinovic O, Pastar I, Tomic-Canic M. Biology and biomarkers for wound healing. *Plastic and reconstructive surgery*. 2016 Sep;138(3):18S.
27. Agyare C, Osafo N, Boakye YD. Biomarkers of wound healing. In *Wound Healing-Current Perspectives 2018 Dec 17* (pp. 24-30). IntechOpen.
28. Hawksworth JS, Stojadinovic A, Gage FA, Tadaki DK, Perdue PW, Forsberg J, Davis TA, Dunne JR, Denobile JW, Brown TS, Elster EA. Inflammatory biomarkers in combat wound healing. *Annals of surgery*. 2009 Dec 1;250(6):1002-7.
29. Hahm G, Glaser JJ, Elster EA. Biomarkers to predict wound healing: the future of complex war wound management. *Plastic and reconstructive surgery*. 2011 Jan 1;127:21S-6S.
30. Goto T, Saligan LN. Wound pain and wound healing biomarkers from wound exudate: a scoping review. *Journal of wound, ostomy, and continence nursing: official publication of The Wound, Ostomy and Continence Nurses Society*. 2020 Nov;47(6):559.
31. Ligi D, Mosti G, Croce L, Raffetto JD, Mannello F. Chronic venous disease–Part I: Inflammatory biomarkers in wound healing. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*. 2016 Oct 1;1862(10):1964-74.
32. Simoska O, Duay J, Stevenson KJ. Electrochemical detection of Multianalyte biomarkers in wound healing efficacy. *ACS sensors*. 2020 Nov 11;5(11):3547-57.
33. Yussof SJ, Omar E, Pai DR, Sood S. Cellular events and biomarkers of wound healing. *Indian Journal of Plastic Surgery*. 2012 May;45(02):220-8.
34. Patel S, Maheshwari A, Chandra A. Biomarkers for wound healing and their evaluation. *Journal of wound care*. 2016 Jan 2;25(1):46-55.
35. Hawksworth JS, Stojadinovic A, Gage FA, Tadaki DK, Perdue PW, Forsberg J, Davis TA, Dunne JR, Denobile JW, Brown TS, Elster EA. Inflammatory biomarkers in combat wound healing. *Annals of surgery*. 2009 Dec 1;250(6):1002-7.
36. Morelli T, Neiva R, Nevins ML, McGuire MK, Scheyer ET, Oh TJ, Braun TM, Nör JE, Bates D, Giannobile WV. Angiogenic biomarkers and healing of living cellular constructs. *Journal of dental research*. 2011 Apr;90(4):456-62.
37. Solati K, Karimi M, Rafieian-Kopaei M, Abbasi N, Abbaszadeh S, Bahmani M. Phytotherapy for wound healing: The most important herbal plants in wound healing based on iranian ethnobotanical documents. *Mini reviews in medicinal chemistry*. 2021 Mar 1;21(4):500-19.
38. Pazyar N, Yaghoobi R, Rafiee E, Mehrabian A, Feily A. Skin wound healing and phytomedicine: a review. *Skin Pharmacology and Physiology*. 2014 Jun 27;27(6):303-10.
39. Tsioutsiou EE, Giachetti D, Miraldi E, Governa P, Magnano AR, Biagi M. Phytotherapy and skin wound healing. *Acta. Vulnologica*. 2016 Sep 1;14:126-39.
40. Malafaia O, Campos AC, Torres O, Goldenberg S. Phytotherapy and surgical wound healing. *Acta Cir Bras*. 2006;21(1).
41. Thangapazham RL, Sharad S, Maheshwari RK. Phytochemicals in wound healing. *Advances in wound care*. 2016 May 1;5(5):230-41.
42. Dey A, Gupta B, De JN. Traditional phytotherapy against skin diseases and in wound healing of the tribes of Purulia district, West Bengal, India. *J Med Plants Res*. 2012 Aug 29;6(33):4825-31.
43. Enoch S, Leaper DJ. Basic science of wound healing. *Surgery (Oxford)*. 2008 Feb 1;26(2):31-7.

44. Schultz GS, Chin GA, Moldawer L, Diegelmann RF. 23 principles of wound healing. *Mechanisms of vascular disease: a reference book for vascular specialists*. 2011:423.
45. Kasuya A, Tokura Y. Attempts to accelerate wound healing. *Journal of dermatological science*. 2014 Dec 1;76(3):169-72.