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**REVIEW ARTICLE**

# WOUND HEALING MECHANISM, INFECTION AND MODELS

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**ABSTRACT**

Wound healing though a natural process, is found to be impaired in some conditions. These require therapeutic agents to accelerate and regain loss of functional tissue. Understanding the mechanism behind the healing process is essential to identify enhancing agents. Several phytocompounds have been studied to possess this activity and in-vivo models have been used to screen such compounds. The models used are expected to simulate the wound environment as in humans. Impairment in healing is a major contribution of microbes that colonise the wound and a wide range of such microbes have been reported in clinical cases. These microbes either act by making wound inflammation chronic or by devastating the wound tissues to increase the severity. Development of wound infection models and biofilms has become valuable tools to comprehend the effect of the colonising microbes on the wound.

**KEYWORDS:** Wound healing, Inflammation, Phytoconstituents, Wound infection.

## INTRODUCTION

Wound healing is a complex process of repairing the anatomically damaged or disrupted tissue. Depending upon the time required for wound healing they are classified as acute and chronic wounds. While in the process of wound healing there are many pathogens which attack the wound and cause severe complications. Wounds may be caused accidentally or may be a result of a disease or surgical intervention. Wound healing starts from the time of injury.

Wounds may actually cause infection not only in the outer region of skin but also in the subcutaneous region which even reaches the organs and blood stream by penetration and cause infection. The wounds caused may have some symptoms like redness, swelling or oedema, production of heat in the place of wound [1]. If a person has a very mild infection by a small wound then it can be treated at home by appropriate cleaning with water or any solution and dressing the wound. In case of a severe wound infection occurring with fever, discharge, red streaks or lesions then immediately proper medication should be taken.

## PREVALENCE

around 5% of the cases [2]. Chronic wounds are more prevalent all over the world covering 1 to 2% of population in developed countries [3]. In developing countries like India, about 52% of patients with diabetes are common with foot infection.

## PHASES OF WOUND HEALING

Lesion produced from the wound discontinues the function of the skin and will undergo the tissue repair process and regeneration of tissue. Wound healing period actually depends upon the type of wound; acute wounds may take around 5 to 10 days to heal, or sometimes upto 30 days. Chronic wound may take months or years to heal. Wound healing actually takes place in three phases namely the inflammation phase, proliferative or tissue formation phase, remodelling phase [4].

### Inflammation phase

The inflammation reaction takes place immediately after the wound has occurred. Homeostasis is maintained before the start of the healing process and this make the wound get separated from the outer environment region by clotting of blood [5]. Immune system plays a major role in defending against the pathogen from entering into the body through the wound. The commensal strain which are

According to studies chronic wounds occur in the form of venous leg ulcers

present in the skin would act as a defence layer by releasing the bacteriocin which will inhibit the pathogen to grow [5]. And also commensals will be responsible for the cluster of memory T lymphocytes at the site of hair follicles. Myeloid cells help in the maintenance of the skin homeostasis. Neutrophils and macrophages are the cells involved in this process [6]. Once injury has occurred the platelets are produced which acts as the blood clotting factor. Then leucocytes are formed primarily followed by the serum protein and fibrin strand formation around the wound. The neutrophils actually make the wound infiltrate and enter into the apoptosis process by which they engulf the debris or microorganism. Also the neutrophils act as the first line of defence in acting against the pathogen entering into the wound region, where the pathogen is engulfed by the neutrophils and are phagocytosed and release the enzyme to further lyse the tissue [6]. Macrophages play a major role in wound healing process which will infiltrate the pathogens from entering into the wound and prevents infection [7]. Phagocytes will help in clearing the necrosis debris. The growth factors are released such that they will act in the upcoming proliferative phase.

**Proliferative or tissue formation phase**

Proliferative or tissue formation process follows the inflammation phase of wound healing. Proliferative phase is also called as the re-epithelialisation phase. In this phase the skin resident cells like keratinocytes, fibroblasts, and endothelial cells are mainly involved. At this stage the formation of the keratinocytes and epithelial cells starts towards the wound region. The keratinocytes will restore the barrier function of the epidermis and myofibrils which are derived from the fibroblasts will actually make the wound region contractile. And this takes around 24 hours. The production of these cells are from adjacent hair follicles and sweat glands. The cytokines and the growth factors initiate the re-epithelialisation process [8]. The process of "angiogenesis" where the formation of new blood cells start takes place. Angiogenesis restores the supply of oxygen. The fibroblasts produce collagen substance for regeneration of the tissue. Angiogenesis process takes place with the activation of the macrophages and epidermis. And further even the growth factors are responsible. The excess neutrophil and macrophages are destroyed in this phase.

**Remodelling phase**

The remodelling phase actually takes place from 7 days to months after the wound. The remodelling phase includes the reformation of the skin back to its original form and scar tissue formation. In this process the immune cells, epithelial cells and the myofibrils undergo apoptosis or programmed cell death [5]. The process requires more proteins, in case of any protein deficiency there will be inhibition of remodelling. In this phase the scar formed will disappear [1]. The myofibroblasts is taking the important role in wound contraction. The collagen is actually reticular and in case of wound it appears unshaped or undefined. Collagen matrix will be present about 3 days after wounding and later disappears [1]. The wound margins contain the growth factors which are released to activate the dermal fibroblasts. Collagen is laid during the proliferative phase, disorganised and the region of the wound becomes thick. The collagen cross links and the scar reduces and makes the wound skin region stronger.

**PHYTOCONSTITUENTS IN WOUND HEALING**

Medicinal plants have many naturally present chemical substances which actually help in healing both the acute and chronic wounds. Phytoconstituents in the plant interact with the wound at various stage of the wound healing and will show positive effect. India is in the 8$^{th}$ position all over the world in traditional medicine practice [9]. A good phytoconstituent to be a wound healing agent, should have the property of anti-inflammatory, anti-microbial, anti-oxidant and analgesic activities.

**Aloe vera**

Aloe vera belongs to the family Liliaceae and is available throughout India. It is a medicinal herb and most effective agent in wound healing [10]. Aloe vera is generally used in second degree wound where the outer layer and its underneath tissue are affected. It is used in healing both the acute and chronic wounds. There are about 100 phytoconstituents present in the Aloe vera. It forms a protective layer on the wound surface and prevents the microorganism from entering into the wound. Meta-analysis of studies with burn wound patients resulted in good wound healing. Aloe vera gel actually helps in increasing the collagen synthesis and formation of new cells and tissues. It also contains polymers and glucomannans or peptic acid. Aloe vera is used for wound healing because of its anti-inflammatory, anti-proliferative, lipid and glucose lowering and antioxidant functions [11].

**Honey**

Honey is the saturated sugar solution obtained from the nectar. Use of honey in wounds is recorded to be an ancient method of curing wounds. It is used generally for curing minor acute wounds. In addition to honey, copaiba oil-resin, a commercial product is given to treat wounds. Honey when used in burn wound showed a greater result showing re-epithelialisation, angiogenesis, granulation tissue formation. It can also provide nutrients and enhance the anti-scar activity. Chronic wounds such as the leg ulcer wounds, sickle cell ulcer, diabetic skin grafting sites, skin lesions are successfully cured using honey. Honey's anti-bacterial activity aids removal of microbial burden on the wound [12]. Bee honey dressing can be done for chronic wound such as diabetic foot ulcers.

**Curcumin**

Certain phytochemicals such as the curcumin which is called as the "Indian Safron" is used to treat wounds as they contain bioactive compounds. It has been identified from *Curcuma longa* belonging to the family Zingiberaceae. It is an Indian spice which is used to cure many ailments. It has anti-inflammatory and anti-oxidant properties which heals the wound. It is very effective than the standard drug Povidone Iodine Ointment [13]. Curcumin nanocomposite wound dressing are used for wound healing as they showed fast drying activity and water absorbency is also low.

**Euphorbia heterophylla**

*Euphorbia heterophylla* which belongs to the family *Euphorbiaceace*, is a medicinal plant used in olden days to treat wounds. It has the property of curing the wound faster than other phytoconstituent. This plant has anti-microbial, anti-fungal, anti-viral, anti-oxidant and anti-inflammatory properties. Phytochemical constituents such as tannins and flavonoids are present which helps in the contraction of the wound and epithelialisation. The extract of *Euphorbia heterophylla* inhibits the growth of microorganisms [14].

**Terminalia chebula**

*Terminalia chebula* is a medicinal plant which is known as the King of plants in Ayurveda. It belongs to the family Combretaceae. It has the anti-inflammatory and anti-oxidant phytochemicals which progress the wound healing. It will increase collagen synthesis in wound healing. It acts as a nutraceutical and has pharmacognostic value. The aqueous extract from the dried fruit of *Terminalia chebula* inhibits nitric oxide synthesis [14]. It helps in the proliferation of keratinocytes and fibroblast. It is used especially for burn wounds in which the skin is remodelled. It improves the immunity and also it is anti-microbial and found to normalise the sugar level.

**IN-VIVO EXPERIMENTAL WOUND MODELS**

Several wound models are being established using the animals to screen and evaluate natural product derived drugs for wound healing potency. Frequently used animals include rabbits, mice, rats, guinea pig, zebrafish and a few insects like drosophila. The animals which are used for wound healing have closer physiology to that of humans. Even large animals such as dogs and also primates can be used as a model for wound healing but this is expensive and housing is difficult. Animal model can be used for both chronic and acute wound especially to establish abrasions, cuts and surgical wounds.

**Rats**

Rats are used as an experimental animal in wound healing. Rat models with second degree burn wounds were given cell based therapy with mesenchymal stem cells [11]. Diabetic wound models have also been established in the rats. Diabetic condition is established in the rat and wound healing between the diabetic and non-diabetic rat would be observed. Mice is used as a model for skin repair. They are actually very effective animal model and so relatively large numbers can be included.

**Pig**

Porcine models have been used to establish chronic wounds especially diabetic ulcers and also guinea pig is used for ischemic model. The hair of the guinea pig is generally removed if detecting for any subcutaneous infection. The application of frog skin to the guinea pig has also shown some effective wound healing, since the amphibian's skin contain a variety of bioactive molecules which has therapeutic activities such as the antimicrobial, anti-inflammatory, proliferation, and differentiation of keratinocytes and fibroblast cells. Second degree burn wounds in porcine models were created to look for dermal reaction [15].

**Frog**

Frog's skin is effective in wound healing process. Generally frog's skin is used as a dressing for wound healing and is a traditional practice tool [16]. They regenerate the normal physiology and pathophysiology of the wounded skin. It promotes proliferation of keratinocytes and fibroblasts. The frog's skin works as an effective antifungal and inhibits the growth of pathogenic microorganisms.

**Rabbit**

Rabbit ear model is used for ischemic wound. As this rabbit have similar physiology close to humans they are used as an animal model and immunological evaluation tool. Hyaluronidase and hyaluronic acid was injected in each of the arm of the rabbit and it was noticed for about 5 days for the reaction to be observed [17]. Ocular surface chemical injuries were established in rabbits. The acute wound healing is tested in rabbit's ear and it mimicked that of human. They are difficult to modify genetically and high cost breeding too. The rabbits ear cartilage is highly vascularised which mimics the human dermis vascularisation.

**BIOFILMS IN WOUND HEALING**

Wounds which do not retain its anatomical structure and function, termed as chronic wounds can be treated with biofilms. Biofilm is an assembly of microbial cells forming colonies which forms a coating on the surface of the wound and also enclosing the extracellular polymeric surface [18]. The property of hydrophobicity is very important in making of biofilms, since they have adhesion property. The polysaccharide coating of the biofilm acts as an adhesive agent to attach the colony towards the surface of wound. The biofilms delay healing process by

producing destructive enzymes and toxins that result in a chronic inflammatory stage in the wound and are found to occur in wounds which have visible necrotising tissue. The epithelialisation and granulation tissue formation are impaired in such cases.

## MICROORGANISMS IN WOUND INFECTION

Wound is colonised by polymicrobial community. Most of these microbes are getting resistant to regular antibiotics, causing impairment in healing.

### Methicillin resistant *Staphylococcus aureus* (MRSA)

Methicillin resistant *Staphylococcus aureus* is a Gram positive cocci. It is most commonly found in the health care centres. This can be acquired by prolonged stay in the hospital environment with open wounds, surgery or even any implants. *Staphylococcus aureus* species is actually sensitive to antibiotics previously but evolutionary changes show its nature to resist antibiotics. Few isolates of *Staphylococcus aureus* are sensitive only to glycopeptide antibiotics such as vancomycin. This methicillin resistant *Staphylococcus aureus* is a coloniser and generally exists in burn wounds [19]. It causes more mortality and morbidity across the global region. A study shows special dressing like nanoparticles incorporated with titanium for organisms like the Methicillin resistant *Staphylococcus aureus*.

### *Acinetobacter baumanii*

*Acinetobacter baumanii* is a Gram negative coccobacilli. It is identified as a coloniser in skin of a normal healthy individual. It causes nosocomial infection and easily transmitted through health care workers. Based on the genome sequencing and DNA-DNA hybridisation 42 species of *Acinetobacter* have been identified [20]. *Acinetobacter pittii* and *Acinetobacter nosocomialis* strains predominantly cause infection in the hospital acquired cases. Polymyxin B and colistin (polymyxin E) are helpful in reducing the bacterial load.

### *Pseudomonas aeruginosa*

*Pseudomonas aeruginosa* is a Gram negative bacteria and considered as one of the most life-threatening bacteria by World Health Organisation. The most significant infections caused by this pathogen are chronic and burn wounds. Interestingly, burn infections caused by *Pseudomonas aeruginosa* often spread rapidly and leads to death within days or weeks, but *Pseudomonas aeruginosa* in chronic wound infections persists for a longer period of time with little associated mortality [21]. About 38% of burn wound infection are caused by *Pseudomonas aeruginosa*.

### *Klebsiella pneumoniae*

*Klebsiella pneumoniae* is a Gram negative bacteria which was first identified by Carl Friedlander in 1882 from the lungs of patients who died from pneumonia. *Klebsiella* species are third most common isolated pathogen from burn wounds. This is the most frequent pathogen causing multidrug resistance (MDR) wound infection [22]. Due to this property, the empirical antibiotic therapy would not suffice the wound infection and leads to extensive surgical debridement.

## WOUND INFECTION MODEL

Animal models are used to depict the in vivo wound infection. Rats, mice, porcine, murine, insect, rodent, guinea pig are generally used as animal models. A porcine model of *Acinetobacter baumanii* infection has been used to study the immunological response during wound healing. The similarity in the skin structure and physiology of wound healing among the pigs and humans make these models very efficient. These models are being used for screening of drugs [23]. Guinea pig is used as a burn wound model. There are different types of animal burn model used, namely Boiling water burn model, Ethanol bath burn model, Gas flame burn model, Pre-heated double brass block burn model, Pre-heated (single) metal plate/bar burn model. Murine models are also as animal model for burn wounds. Animal organs like spleen and liver and biological samples like blood and intraperitoneal fluids are used for studying microbial load [24].

## CONCLUSION

Wound healing, being a complex process of repairing the damaged tissue, involves numerous cellular and molecular mechanism. Understanding the nature of wound healing and the modifiable parameters would help to develop better drugs accelerating the process and favourable wound management strategies. More detailed comprehension of the topics covered in the review would be essential for delineating unrevealed mechanisms.

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