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Different bioactive molecules in the process of wound healing: A review

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Abstract
Wound healing is a natural process. However, all the wounds are not heal precisely in the same manner due to differences in the etiology of the wound, presence or absence of infection, and medical or surgical interventions. The primary aim of wound healing is to restore tissue architecture and homeostasis. Different molecules including different cytokines play different role for early haling of wound. At the wound site, cytokines and inflammatory mediators are released that regulates wound healing through control of cell growth and migration, differentiation and proliferation.

Keywords: Wound, healing, cytokine, cell growth and migration

Introduction
The skin is one of the largest organ of the body. In normal skin, the epidermis (outermost layer) and dermis (inner or deeper layer) of skin exist in steady-state equilibrium, forming a protective barrier against the external environment. Once the protective barrier is broken, a wound will be inflicted (Shai and Maibach, 2005; Nagori and Solanki, 2011) [36, 27]. Wound is the disruption of the cellular and anatomic continuity of a tissue that may be produced by physical, chemical, thermal, microbial or immunological insult to the tissue (Robson *et al*., 2001; Atiyeh *et al*., 2002; Raina *et al.,* 2008) [31, 3, 29].

Based on the nature of the repair process, wounds can be either acute or chronic. Acute wounds are typically tissue injuries caused by cuts or surgical incisions that complete the wound healing process within the expected time frame (Strodtbeck, 2001) [39]. In contrast, chronic wounds are wounds that have failed to progress through the normal stages of healing and therefore, enter a state of pathologic inflammation. As a result, the healing process is delayed, incomplete, and does not proceed in a coordinated manner, subsequently resulting in poor anatomical and functional outcome (Menke *et al.,* 2007) [22]. Chronic wounds cause a major disability and are characterized by chronicity and frequent relapse (Menke *et al.,* 2007) [22].

Wound healing is a natural and spontaneous phenomenon (Franklin and Dawson, 2008) [12], but not all wounds heal precisely in the same manner due to differences in the etiology of the wound, presence or absence of infection, and medical or surgical interventions. The primary goal of wound healing is to re-establish homeostasis and restore tissue architecture. Tissue damage triggers a cascade of events aimed at rapid repair (Savanth and Shah, 1998; Martin and Leibovich, 2005) [33, 19]. The process of wound healing may be best understood by dividing it into phases: homeostasis, inflammation, proliferation and remodeling (Midwood *et al.,* 2004; Janis *et al.,* 2010) [23, 17]. These phases are somewhat arbitrary, as they overlap in time, physiology, and cell type, with each phase not entirely completed before the next begins (Molnar, 2007) [25].

The inflammation stage begins immediately after injury that last for 0-3 days, first with vasoconstriction that favors homeostasis and releases inflammatory mediators. Platelets coalesce within minutes to stop the bleeding and begin clot formation. The vasodilation and increased capillary permeability cause inflammation (redness, heat, swelling and pain)**.** An influx of polymorphs and macrophages defend against bacteria, ingest debris and begin the process of repair with neutrophils, the macrophages attract fibroblasts and influence the growth of new blood vessels into the wound by chemotactic activity and the release of potent growth factors and cytokines which activate keratinocytes, fibroblasts, and endothelial cells (Broughton, 2006; Ramasastry, 2005; Diegelmann and Evans, 2004) [5, 30, 10].

Once the inflammation decreases due to the actions of neutrophils and macrophage, their numbers are reduced and, as a result, the proliferation phase is initiated. The proliferative phase is the second stage of wound-healing that last for 3-24 days and includes: epithelialization, neoangiogenesis, formation of granulation tissue and extracellular matrix, and re-epithelialization (Singer and Clark, 1999; Schreml *et al.,* 2010) [37, 34]. There is extensive growth of epithelial cells under the scab that bridges the wound. With the developing new blood vessels multiplication of the fibroblasts occurs. Collagen strands are deposited in a haphazard way and form a fibrous network that supports the new capillary loops. The tissue formed is granulation tissue with a moist translucent red appearance. Signs of inflammation disappear and the fibroblasts contract pulling the wound edges together. The tensile strength of the wound is increased during this stage and this process continues into the next phase, the maturation stage.

During the maturation phase which last for 24 days to 1 year, fibroblasts leave the wound and collagen is remodeled into a more organized matrix. This changes the appearance from red granulation tissue to a pink early epithelialization. Finally a white relatively avascular tissue develops, and the epidermis is restored to normal thickness. Tensile strength increases for up to one year following the injury. While healed wounds never regain the full strength of uninjured skin, it can regain up to 70% - 80% of its original strength. Remodeling is thus a balance between the synthesis of new collagen and the degradation of old (Robson *et al.,* 2001) [31]. As the wound healing process is switched off, the new connective tissue matures and changes from pinkish-red to a white color (Davis and Senger, 2005) [9].

A wound may lead to serious consequences if not treated at proper time (Harpal and Kuldip, 1993; Mashhood *et al.,* 2006) [15, 21]. In a non-healing chronic wound, the healing process has been disturbed and is often detained in an inflammatory phase, and unable to progress into the next stage of cell proliferation. Wound healing of injured skin and other tissues is fundamental for survival. Successful wound healing requires timely and optimized function of many different cell types, structural elements, molecular mediators, and processes. Disturbances of any of these functions result in impaired healing (Guo and Dipietro, 2010) [14].

Skin wound treatment is a very diverse part of the health care system, encompassing surgical and accidental lacerations, burns, pressure ulcers, diabetic and venous ulcers. The treatment of wounds and associated complications (Chronic and nonhealing wounds) are especially costly because they require repetitive treatments (Beckrich and Aronovitch, 1999) [4]. Stem cell therapy is the use of stem cells to treat or prevent a disease or condition. Research is underway to develop various sources for stem cells, and to apply stem cell treatments for neurodegenerative diseases and conditions, diabetes, heart diseases and other conditions.

While engineered skin substitutes represent significant advances in wound care, their use is not routine because of their high cost, limited effectiveness and their inability to reconstitute skin appendages. Stem cells, as defined by Ernest A. McCulloch and James E. Till**,** are characterized by prolonged self-renewal capacity and the ability to differentiate into mature stages and different tissue types by asymmetric replication. Stem cells, due to their ability to differentiate into various tissue types by asymmetric replication, may help create those skin components that are not found in the tissue engineered skin substitutes. Among the main sources of cells that might be used for repair and regeneration of injured skin are adult stem cells, embryonic stem cells (ESCs) and induced pluripotent stem cells (iPS) cells.

Stem cell treatment is a type of cell therapy that introduces new cells into damage tissue in order to treat a disease or injury. Medical researchers believe that stem cell therapy has the potential to dramatically change the treatment of various diseases. A number of adult stem cell therapies already exist, particularly bone marrow transplants that are used to treat leukemia. Bone marrow transplant is a crude form of stem cell therapy that has been used clinically for many years without controversy. No stem cell therapies other than bone marrow transplant are widely used.

While it is well-known that during the inflammatory phase of wound healing, blood-borne immunocompetent cells invade the wound area, recent evidence suggests that bone marrow-derived stem cells are also recruited into the wound site (Cottler-Fox *et al.,* 2003; Fu and Liesveld, 2000) [8, 13]. This is not completely surprising, since a small number of hematopoeitic and mesenchymal stem cells are always present in peripheral blood. Furthermore, severe injury has been shown to increase the number of circulating stem cells (Kucia *et al.,* 2004) [18]. Bone marrow derived mesenchymal stem cells contribute to the reconstitution of the dermal fibroblast population in cutaneous wounds (Fathke *et al.,* 2004) [11]. These findings suggest a potential important contribution of stem cell homing to the wound healing process, which is currently not well understood and warrants further study.

At the wound site, cytokines and inflammatory mediators are released that regulates wound healing through control of cell growth and migration, differentiation and proliferation (Hubner and Werner, 1996 and Salvin, 1996) [16, 32]. Cytokines are a group of soluble protein or polypeptide mediators that have profound effect upon host immune responses and are the mediators of inflammation. Cytokines may be proinflammatory (IL-1, TNF-α etc.) or anti-inflammatory (IL-1 antagonist like IL-4, Il-10) on the basis of their functions.

Nutrition has been recognized as a very important factor that affects wound healing. Energy, carbohydrate, protein, fat, vitamin, and mineral metabolism all can affect the healing process (Arnold and Barbul, 2006) [2]. Together with fats, carbohydrates are the primary source of energy in the wound-healing process. Glucose is the major source of fuel used to create the cellular ATP that provides energy for angiogenesis and deposition of the new tissues (Shepherd, 2003) [36]. The use of glucose as a source for ATP synthesis is essential in preventing the depletion of other amino acid and protein substrates (Arnold and Barbul, 2006) [2]. Protein is one of the most important nutrients affecting wound healing and its deficiency can impair capillary formation, fibroblast proliferation, proteoglycan and collagen synthesis, and wound remodeling (Campos *et al.,* 2008) [7].

Collagen synthesis requires hydroxylation of lysine and proline, and co-factors such as iron and vitamin. Collagen and elastin are the predominant extracellular protein of granulation tissue in a healing wound and there is a rapid increase in its synthesis in the wound area soon after an injury. Breakdown of collagen liberates free hydroxyproline and its peptides. Measurement of hydroxyproline has been used as an index of collagen turnover. Hexosamine and hexuronic acids act as ground substratum for the synthesis of new extracellular matrix.

Uric acid, a metabolic product of purines, may exert a role in tissue healing. It acts as an alarm initiating the inflammatory process that is necessary for tissue repair, as a scavenger of

oxygen free radicals and mobilizer of progenitor endothelial cells.

Several micronutrients have shown to be important for optimal repair. Magnesium functions as a co-factor for many enzymes involved in protein and collagen synthesis, while copper is a required co-factor for cytochrome oxidase, for cytosolic antioxidant superoxide dismutase and for the optimal cross-linking of collagen. Zinc is a co-factor for both RNA and DNA polymerase and is necessary for tissue regeneration and repair. Zinc deficiency causes a significant impairment in wound healing. Iron is required for hydroxylation of proline and lysine, and, as a result, severe iron deficiency can result in impaired collagen production (Shepherd, 2003; Arnold and Barbul, 2006; Campos *et al.*, 2008) [36, 2, 7].

At the same time, antioxidants play an important role in protecting the body against reactive oxygen species (ROS) (Martinez-Maqueda *et al.*, 2012) [20]. Over production of ROS results in oxidative stress thereby delayed wound healing. Free radicals are highly reactive molecules derived from the normal metabolism of oxygen, or from exogenous factors and agents. Free radical scavenging enzymes has an essential role in the reduction, deactivation, and removal of ROS, as well as regulating the wound healing process. Imbalance in free radicals (such as Nitric oxide, lipid peroxidation etc.) generations and antioxidants (like GSH, SOD, and CAT) production has been observed to induce oxidative stress and tissue damage, thereby delayed wound healing. Therefore, elimination of reactive oxygen species could be an important strategy in wound healing (Mikhalchik *et al.*, 2006; Murthy *et al.*, 2013) [24, 26]. Chronic wound undergoes substantial oxidative stress by neutrophils-derived oxidants and myeloperoxidase activity, both of which contribute markedly to tissue damage during chronic wound inflammation (Song *et al.*, 2008) [38].

Vitamins A, C and E show potent antioxidant and anti-inflammatory effects. Vitamin C and A have many roles in wound healing, and a deficiency in these vitamins have multiple effects on tissue repair. Vitamin E, an antioxidant, maintains and stabilizes cellular membrane integrity by providing protection against destruction by oxidation. Vitamin E also has antiinflammatory properties and has been suggested to have a role in decreasing excess scar formation in chronic wounds (Arnold and Barbul, 2006; and Burgess, 2008) [2, 6].

Many allopathic drugs are known to inhibit wound healing. Topically applied steroids inhibit fibroblast proliferation and collagen synthesis and may cause peripheral vasoconstriction at the wound interface (Sussman, 2007) [40]. Antiplatelet drugs and other non-steroidal antiinflammatory drugs impair wound healing by inhibiting prostacyclin synthesis, inflammatory mediators derived from arachidonic acid metabolism and platelet aggregation. Despite tremendous advances in the pharmaceutical drug industry, the availability of drugs proficient of stimulating the process of wound repair is still limited (Udupa *et al.*, 1995) [42]. Moreover, the management of chronic wounds is another major problem due to the high cost of therapy and the presence of unwanted side effects.

The main goal of wound management is rapid wound closure and a functional aesthetic scar. Bone marrow seems to be a logical candidate for the treatment of wound or injury as it contains inflammatory cell progenitor, mesenchymal stem cells and multipotent stem cells. Inflammatory cells and haematopoietic hormones have been reported to accelerate wound healing; mesenchymal cell fills the dermis of the skin.

A good deal of exploration has been done regarding treatment of wound by stem cells, by various researchers whose findings suggest the potential important contribution of stem cells in wound healing process, which is till now, not clearly understood and warrants further study in both human and animals. As external violence produces open wound in the skin and are more common in ruminants (Nooruddin and Dey, 1990) [28]. Animals can get wounded at the farm, during transportation, or by getting strike against some hard object, kicked by other animal and during different surgical interventions.

Currently, rabbit Mesenchymal stem cells are very popular among researchers due to their resemblance in cellular and tissue physiology with that of human MSCs (Warden, 2007) [43] and due to their easy availability. To evaluate stem cells, it is important to have uninterrupted source of such cells that too with minimal ethical issues. Rabbits are readily available with larger size compared to mouse or rat and easy to handle and cost effective in comparison to dog, sheep or goat (Tan *et al*., 2013) [41]. However, the literature about the basic characteristics of rabbit MSCs and its application is very scanty in comparison to human Mesenchymal Stem Cells (hMSCs) (Warden, 2007 and Amini *et al*., 2012) [43, 1]. As seen from this brief review, wound-healing is a complex process in which many different cell types, processes and factors are involved.

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