

## **CHAPTER 4: MICROSCOPIC EVALUATION OF WOUND HEALING**

### **4.1 INTRODUCTION**

The rapid expansion of knowledge of the wound healing process has led to the production of more advanced wound care products in the past two decades. Although, these new products have leads to the improvement of the ability to heal the wounds but there are still with some complications (Monaco and Lawrence, 2003).

The different types of acute skin wounds, including surgically created wounds, partial thickness injuries, burns, and traumatic wound, involve significant tissue loss and wound healing duration which could take time. Although the wound healing process varies among the different types of wounds, there are more similarities than differences between these healing process (Monaco and Lawrence, 2003; Li et al., 2007).

#### **4.1.1 ACUTE WOUND HEALING**

Acute wound healing normally involves three distinct but overlapping phases, namely inflammatory phase, proliferative phase and remodeling phase (Singer and Clark, 1999; Monaco and Lawrence, 2003; Li et al., 2007). Some literatures divided the healing process into four stages, with hemostasis is the initial stage (Monaco and Lawrence, 2003; Diegelmann and Evan, 2004; Li et al., 2007).

Bleeding occurs immediately after tissue injuries while hemostasis started on 0 to 48 hours after injuries (Halloran and Slavin, 2002; Li et al., 2007). Hemostatis includes vasoconstriction, platelet aggregation and fibrin deposition resulting in the clot formation (Monaco and Lawrence, 2003). Platelets are the first to appear after injury. These organelles get activated when exposed to the extracellular matrix in the vascular

wall. It also contributes to the other processes in wound healing, which are the inflammation, reepithelialization, fibroplasias and angiogenesis (Diegelmann and Evan, 2004; Li et al., 2007).

At the beginning of inflammatory phase, neutrophils and monocytes are predominant at the wound site. They migrate from capillaries into the wound tissue. Neutrophils are the first cells to arrive in great numbers and their number declines after some times. The tissue derived monocytes (macrophages) then predominate the wound tissue. Neutrophils are the cells that kill and phagocyte the bacteria and damaged matrix proteins within wound bed. The infiltration of neutrophils only last for a few days in normal wound (Li et al., 2007).

Monocytes are transformed into phagocytic macrophages at the wound site. Macrophage is an important regulatory cell in the inflammatory reaction. It phagocytizes, digests and kills the pathogenic organisms. Besides that, it also scavenges the tissue debris and destroys the remaining neutrophils. These processes subsequently allow angiogenesis and formation of granulation tissue. The macrophages are important in the transition between the inflammations and repair processes because it releases chemotactic factors (cytokines) that attract fibroblasts and endothelial cells to the wound area for the development of granulation tissue. It also helps in the growth of the new blood vessels (Halloran and Slavin, 2002; Li et al., 2007). The inflammatory response to the injury provides an important framework to the proliferation. Inflammatory cytokines function in regulating angiogenesis. Proliferation of epithelial cells is followed by migration toward the midline to reform a thin epidermal layer under the surface of the clot (Halloran and Slavin, 2002).

On day 3 to day 5 of post injury, proliferation (epithelialization) continues under the scab with subsequent surface keratinization. This phase may last for 2 to 4

weeks after wounding. It is mainly characterized by fibroblasts migration, deposition of the extracellular matrix and formation of granulation tissue (Halloran and Slevin, 2002; Enorch and Leaper, 2007).

Fibroblast migration appears in the wound on day 2 to day 4 after wounding. After injury, fibroblasts are attracted to the wound by cytokines. They then proliferate and produce the matrix proteins fibronectin, hyaluronan and collagen. The components formed help to construct the new extracellular matrix (ECM), which is important for the regulation of further synthesis of extracellular matrix and remodeling (Enorch and Leaper, 2007).

Collagen is synthesized by fibroblasts. It is the most abundant protein in human body. This component provides the strength and integrity to all the tissue, especially the healed wound. Collagen synthesis occurs during the proliferation and remodeling phases (Enorch and Leaper, 2007). The metabolism of collagen is dynamic with a constant flux between synthesis and degradation, and this allows collagen remodeling (Halloran and Slavin, 2002).

Granulation formation is also found in this phase. Granulation tissue has a pink, soft, granular gross appearance. It is characterized by angiogenesis. This tissue bleeds easily if traumatized and the appearance may indicate wound status (Enorch and Leaper, 2007).

Angiogenesis or neovascularization is the formation of new blood vessels (Singer and Clark, 1999; Enorch and Leaper, 2007). It becomes active from day 2 after wounding (Monaco and Lawrence, 2003). This new blood vessel is to sustain the newly formed granulation tissue (Singer and Clark, 1999). This process is important in providing nutrients and oxygen to the growing tissues and aids in the formation of granulation tissue (Shaw and Martin, 2009).

Epithelialization occurs within few hours after wounding. A single layer of the epidermal cells migrates from wound edges to form a delicate covering over the exposed area. After about 12 hours, there will be an increase in mitotic activity within the basal epithelial cells of the wound edges (Enorch and Leaper, 2007).

The synthesis and remodeling of the extracellular matrix is initiated with the development of granulation tissue and continues for prolonged periods. Continuous synthesis and breakdown of collagen occur during this phase. The interaction between fibroblasts and surrounding extracellular matrix will result in the wound contraction (Enorch and Leaper, 2007). Wound contraction peaks at 2 weeks after wounding. Myofibroblast is the predominant mediator of this contractile process (Li et al., 2007).

The transition of granulation tissue to scar involves reorganization and maturation of collagen fibers. Delicate balance of collagen synthesis and collagen degradation is important in this stage of healing. In normal wound, the collagen synthesis and breakdown is achieved within 3 weeks to 2 years post-injury (Enorch and Leaper, 2007).

#### **4.1.2 HISTOLOGICAL ASSESSMENT OF WOUND HEALING**

The *in vivo* wound model enables a longitudinal temporal assessment of the complicated wound healing process. This type of model enables us to study the contribution of the overlapping but distinct processes of wound healing. Thus, the insight gained from the wound research through investigation of healing process in both time and stage specific manner is expected to contribute to the development of novel therapies (Braiman-Wiskman et al., 2007).

The histological approach is normally in wound study used in evaluating the efficacy of wound treatments during wound healing (Ukong et al., 2008). There are several wound characteristics used as histopathological parameters to determine the progress of the wound healing process. These parameters are the depth and length of healed wound, epithelial stratification, incorporation of the dermal substitute and , degree of neutrophils, macrophages, fibroblasts and elastin formation (Truong et al., 2005).

The wound healing process can be visualized by studying the functional parameters and structural changes of the skin (Braiman-Wiskman et al., 2007; Ukong et al., 2008). These parameters include the epidermal closure, granulation tissue formation, inflammation, dermal closure and matrix remodeling (Braiman-Wiskman et al., 2007). All of these parameters could also be analyzed by using histological approach. The following table shows the staining methods used to identify certain specific features in wound healing.

**Table 4.1:** Histological cell parameter for wound healing assessment (adapted from Braiman-Wiskman, 2007).

<b>Staining Method / Marker</b>	<b>Healing Parameter</b>	<b>Assessment Parameter</b>
Hematoxylin & Eosin (H&E)	Inflammation Dermal Closure	White blood cells Abscesses matrix remodeling
Masson's Trichrome (MT)	Granulation Tissue formation and matrix formation	Collagen fiber deposition
Verhoeff's Elastic (VE)	Late stage of matrix remodeling	Elastic fiber deposition.