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Review Article

WOUND HEALING: A SHORT REVIEW

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Abstrct: Wound healing is a complex process and it is the result of cell-cell interactions and cell-matrix interactions. The different phases of wound healing are controlled by these interactions and growth factors that are released in phase specific manner. In certain predisposed individuals, these processes are not regulated in phase specific manner due to lack of timely signals and normal process of wound healing is disturbed which may result into scar formation such as hypertrophic scars and keloids. The factors involved in abnormal wound healing are addressed in the present review.

Key words: wound healing,

Wound, the damage caused by environmental insults such as mechanical and chemical injuries, results in the disruption of the normal anatomical structure and function [1]. This may extend from the epidermis deep into the muscles depending on the severity of damage. Wound thus caused can be healed by a spontaneous process in the organism through a cascade of events, which starts by switching on various chemical signals in the body; thus facilitates the restoration of anatomical continuity and function. Wound healing is a highly complex process. The interaction of a variety of different cell types namely keratinocytes, endothelial cells, fibroblasts and inflammatory cells which communicate by direct cell-cell interaction or by cell-matrix interaction or by chemical signals in the form of cytokines are some of the steps for normal wound healing. While partial thickness wound heals by mere epithelialisation, the healing of full thickness wound which extends through the entire dermis involves more complex well-regulated biological events [2].

The healing process begins with the clotting of blood and is completed with remodelling of the cellular layers of the skin. The whole process can be broadly be classified into 5 overlapping phases namely

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inflammation, granular tissue formation, reepithelialisation, matrix production and remodelling [3].

The coordination of different variety of cells, release of growth factors and the signals for proliferation and differentiation play a crucial role in each of these phases [4-7]. The cells which take part during healing process are inflammatory cells, fibroblasts, endothelial cells and keratinocytes. Despite the minute quantity, the growth factors control and regulate the proliferation, differentiation and metabolism of the cells in each of the phases during wound healing. The growth factors bind to the receptor site at the cellular surface and act in a paracrine, autocrine or endocrine fashion according to the message delivered to carry out the processes. Thus the coordinated interaction among cells, growth factors and matrices becomes essential for normal healing.

As blood spills into the site of injury, the blood components and platelets come in contact with exposed collgen and other extracellular matrix components. This contact triggers the release of clotting factors from the platelets and the formation of a blood clot, composed of biologically active substances such as fibrin, fibronectin, vitronectin and thrombospondin [1,8-10]. The blood clot also provides a provisional matrix for cell migration in the subsequent phases of the haemostatic and inflammatory phases. The cytoplasm of platelets contains α-granules filled with growth factors and cytokines, such as platelet derived growth factors (PDGF), transforming growth factor-β (TGF-β), epidermal growth factor and insulin-like growth factors [9].These molecules act as promoters in the wound healing cascade by activating and attracting neutrophils and later, macrophages, endothelial cells and fibroblasts [9,11].

Inflammatory phase: The neutrophils play an important role in phagocytosis in order to destroy and remove bacteria, foreign particles and damaged tissue to prevent infection [1,12,13]. Upon completing the task, the neutrophils are removed by macrophages as slough and by apoptosis without tissue damage or potentiating the inflammatory response [13,14]. Macrophages are the predominant inflammatory cells [15] and are derived from the activated peripheral monocytes normally present in the connective tissue matrix [2]. They induce the beginning of the granulation phase by the release of various growth factors that stimulate the wound fibroblasts, make them mobile and make them migrate to the area of injury [16,17]. They also control wound healing through the production of growth factors such as PDGF, TGF-α, TGF-β, interleukin (IL-1) bFGF and tumour necrosis factor (TNF) [18,19]. Macrophages also help in angiogenesis by secreting growth factors such as TGF-α and TGF-β which stimulate angiogenesis which is an essential event during wound healing. Stimulated by certain angiogenic factors, the endothelial cells are activated and they degrade basement membrane. Thus macrophages have a longer lifespan than neutrophils and continue to work at a lower pH [20,21].

The last cells to enter the wound site in the inflammatory phase are lymphocytes, attracted by the action of interleukin-1 (IL-1), which plays an important role in the collagenase regulation, which is needed for collagen remodelling, production of extracellular matrix components and their degradation [13,22,23].

Proliferative phase: The proliferative phase is characterised by fibroblast migration and deposition of newly synthesised extracellular matrix, acting as a replacement for the provisional network composed of fibrin and fibronectin. Fibroblasts migrate into the wound, being attracted by factors such as PDGF, TGF-β. They proliferate profusely and produce matrix proteins hyaluranon, fibronectin, proteoglycans, type I and type III procollagen. [1,20,24]. Having accomplished the task, redundant fibroblasts are eliminated by apoptosis [25,26,27]. Synthesised by fibroblasts collagens impart integrity and strength to all tissues and play a key role, especially in the proliferative and remodelling phases of repair [26-28]. While unwounded dermis contains 80% type I and 20% type III collagen, wound granulation tissue expresses 40% type III collagen [1].

Granular tissue formation; The major events of granular tissue formation are ingrowth of the fibroblasts and deposition of a loose ECM composed of collagen, fibronectin and proteoglycans in particular hyaluronate. The synthesis of ECM is influenced by a variety of fibrogenic growth factors such as PDGF, TGF-β, IGF-1 and bFGF [29,30]. These fibrogenic growth factors upregulate ECM protein production and inhibit the production of proteases in order to maintain the balance between

production and degradation. Macrophages, proliferating fibroblasts together with collagen matrix, fibrinogen, fibronectin and hyaluronic acid constitute the acute granulation tissue that replaces the fibrin – based provisional matrix [24,26] with collagen accumulation and the granulation tissue gradually matures to produce a scar.

Re-epithelisation: To cover the granulation tissue thus formed, the keratinocytes undergo a process called reepithelialisation. Keratinocytes are activated by exposure to hydrogen peroxide, pathogens, growth factors and cytokines [31]. This activation causes keratinocytes at the wound edge to undergo partial epithelial-mesenchymal transition where they develop more invasive and migratory phenotype [32]. Front-to-rear polarity replaces top-to-bottom polarity, allowing the leading edge keratinocytes to migrate laterally across the wound surface in the absence of basement membrane that has been destroyed during wound injury. [33].

The wound keratinocytes have been shown to synthesise most of the basement membrane components such as type IV collagen, Laminin and type VII collagen and regenerate basement membrane [34]. The basement membrane at the dermal-epidermal junction (DEJ-BM) provides the interface between the dermis and epidermis consisting of a highly organized assembly of glycoproteins and proteoglycans and excerts several important functions. It acts as a permeability barrier that controls exchange of macromolecules. Repair of Dermis epidermis junction and basement membrane (DEJ-BM) during wound healing is important for restoration of skin functional properties after wounding. Laminin-$\gamma 2$ is expressed continuously by migrating keratinocytes during re-epithelialisation, whereas collagen IV and collagen VII are deposited after wound closure. However, DEJ-BM restoration following wounding is deficient in elderly individuals. During wound healing only Laminin-$\gamma 2$, but neither Collagen IV nor collagen VII was detected near keratinocytes forming the new epidermis. [35]. While collagen and fibronectin perform the role of signalling the keratinocytes migrate, laminin serves as a stop signal for the migration. It has also been demonstrated that within the reformed basement membrane (dermal-epidermal junction) laminin did not appear until the keratinocytes had stopped migrating and had resurfaced the wound [36]. The regeneration of basement membrane also includes the formation of hemidesmosomes and anchoring fibrils. Anchoring fibrils are thread like structures that link keratinocytes hemidesmosome adhesion complexes to the lamina densa, the basement membrane layer below the lamina lucida. Although anchoring fibrils numbers are comparatively less in normal skin, they are very much essential for the integrity of dermal-epidermal junction.

MMPs (zinc containing proteins), particularly MMP-1 and MMP-9 are vital for keratinocyte migration [33]. The production of other proteases such as plasmin further facilitates keratinocytes migration by degrading the provisional fibrin-rich wound bed [37]. When keratinocytes from opposing edges meet, migration terminates, a thin epithelial layer is established and keratinocytes form new adhesions to the underlying matrix. Keratinocytes then fully reform the basement membrane and undergo terminal differentiation, to stratify and regenerate the epidermis [26]. The process of reepithelialisation occurs until thickened mature skin covers the wound [19]. Once re-epithelialisation is completed, keratinocytes resume their normal differentiated form [38]. Keratins have been widely used as molecular markers for the differentiation of keratinocytes [39].

Remodelling: As the final phase of wound healing, the remodelling phase is responsible for the development of new epithelium and final scar tissue formation. The remodelling of connective tissue involves both the coordinated synthesis of new matrix components and the degradation of Extracellular matrix [ECM]. Both pathways are maintained under tight control and equally balanced. This process reduces cell migration and cell differentiation. The turnover of the matrix is modulated by a number of biological events. These steps require extensive degradation of the ECM components including interstitial collagen, basement me mbrane collagen (type IV), fibronectin, laminin and various proteoglycans [40]. These events are accomplished by MMPs (Matrixmetalloprotinases which are zinc containing enzymes) produced by neutrophils, macrophages and fibroblsts in the wound. Their activity is tightly regulated and synchronised by inhibitory factors. Gradually the activity of tissue inhibitors of MMPs increases, culminating in the activity of metallo proteinases thereby promoting new matrix accumulation [28,41,42].

The organization of collagen bundles, which are highly disorganised in the initial stage becomes more oriented and cross linked over time. It is achieved at the final stages of remodelling phase, to a great extent by the wound contraction. The process is regulated by a number factors, with PDGF, TGF-β and FGF being the most important [28,43]. With time, density of fibroblasts and macrophages is further reduced by apoptosis [27,44]. The end result is a fully matured scar with a decreased number of cells and blood vessels [45-47].

Remodelling in abnormal wounds: While in normal wound healing these processes are in a well organised manner, in certain predisposed individuals the process go awry, the cause for which still remain elusive. The alterations in these process can result either in poor healing leading to chronic wounds or excessive healing resulting in hypertrophic scars and keloids which are unique to human. Both hypertrophic scars and keloids are raised scars, hypertrophic scars remain within the boundaries of the wound and regresses spontaneously, keloids extend beyond the wound margins and rarely regress [48-52].The wound healing sequence did not differ in abnormal wound healing markedly from that seen in normal scars [49], however, cell-matrix interactions has major effect and disruption in this interaction instead of healing the wound by cell proliferation etc., causes apoptosis. Therefore this favours the abnormal wound repair [53]. Either excessive synthesis of collagen, fibronectin and proteoglycan by fibroblasts or deficient matrix degradation and remodelling may be the possible causes for the formation of abnormal lesions such as hypertrophic scar and /or keloid [54,55].

Morphological studies on keloids revealed an alteration in the organization of collagen bundles. While in normal skin the collagen bundles are arranged in a way parallel to the epithelial surface, in keloids, they are loosely packed, leading to haphazard orientation [48]. Abundance of collagen fibres of similar diameter in both reticular dermis (RD) and the papillary dermis (PD) was also observed in keloids compared to normal skin [56]. Disordered packing of epidermal layers and gaps in the basement membrane of keloid tissue was observed. The hemidesmosomes and anchoring fibrils are considerably reduced in numbers in keloid tissue when compared to normal skin [57] and altered shape of desmosomes in the entire enlarged spinous layer was also observed [56]. Keloid keratinocytes exhibit adhesion abnormalities and display a transcriptional signature reminiscent of cells undergoing epithelial-mesenchymal transition [58].

Human fibroblasts from hypertrophic scar tissue respond differently to EGF and TGF-β. when compared to normal fibroblasts.It has also been reported that TGF and their receptors are differently regulated during normal and impaired wound healing. The expression of TGF-beta (TGFss) TGFss 1,2,3 and their receptors in keloids and hypertrophic scars showed that significantly lower TGFss2 mRNA expression in hypertrophic scars as compared with fibroblasts derived from keloids and normal skin. In contrast TGFss3 mRNA expression was significantly lower in keloid fibroblasts in comparison with fibroblasts derived from hypertrophic scars and normal skin. TGFssh1mRNA decreased in hypertrophic scar fibroblasts and TGFh2 decreased in keloids compared with hypertrophic scars [59].

Glycosaminoglycans (GAG) are also present in increased amounts in keloids [61]. The rates of fibronectin gene expression was found to be increased as much as three fold in keloid when compared to normal fibroblasts [60]. The distribution of hyaluranon (HA) was found to differ between the various scar tissues. In normal skin an intense HA staining was observed in the papillary dermis. In mature scar tissue the distribution of HA resembled that of normal uninjured tissue, but the layer of HA was thinner. In hypertrophic scar tissue, HA occurred mainly as a narrow strip in the papillary dermis. Keloid tissue showed the least HA staining of the papillary layer and resembled that of the bulging reticular dermis [61].

The Pro inflammatory factors such as interleukin IL1-alpha, IL1 beta, IL-6 and TNF- alpha are up regulated in keloid tissue [62]**.** Studies carried out on the keratin expression have revealed an abnormal increase in the 58 and 50 kDa keratin protein expression (corresponding to K5 and K14 keratins) in keloids compared to hypertrophic scars and normal skin [63].

CONCLUSION

Wound healing remains a challenging process which involves continuous phases starting from coagulation and ending in the formation of normal scar. However, in certain individuals this results in the formation of hypertrophic scars and keloids the cause for which

still remains elusive. Further studies of the events involved in different phases may throw more light and help the wound management.

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