Periodontal Wound Healing and Cytokines

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Abstract

Wound healing is a highly ordered and well-coordinated process. It involves several sequential phases of inflammation, haemostasis, proliferation, granular tissue formation, matrix formation and remodeling of the injured tissue. It is characterized by dynamic and reciprocal interactions among components of the extracellular matrix, growth factors, and cells. Although the proper healing of periodontal tissues is regulated by many factors like blood cells, epithelial and connective tissue cells, inflammatory cells and many soluble factors, mainly coagulation factors, growth factors and cytokines, the molecular mechanisms involved in the process of wound healing, still remain unclear. The interaction between periodontal pathogens and inflammatory process is regulated by a sequential network of cytokines and it is essential for most periodontal tissue breakdown, leading to clinical signs of disease. The cytokine network takes control over inflammatory mechanisms in order to amplify or suppress tissue reactions in periodontal pathogenesis. Extracellular matrix macromolecules or some of their specific domains may play a major role in wound healing. Enhanced knowledge of these relations may suggest new therapeutic targets in wound healing process. In this review, mainly the pathophysiology of periodontal wound healing is highlighted.

Keywords: Cytokines; Inflammation; Interleukins; Periodontitis; Wound healing

Introduction

Periodontitis is the most common diseases in humans, characterized by progressive destruction of the tooth-supporting tissues. Apart from tooth desorption, periodontitis has been linked to many systematic disorders, such as diabetes, coronary artery disease and stroke, as well as high risk of premature birth [1]. Diabetes mellitus (DM) is a disorder that affects millions of people worldwide. The risk of the development of periodontitis is substantially greater in people with T2DM than in the general population [2]. Periodontitis has been identified as a possible risk owing to poor metabolic control in patients with T2DM. During tooth extractions, there is a great risk of post-operative complications from impaired wound healing and thus the necessity to apply procedures that can accelerate and foster the healing process [3].

Wound healing process involves the activation of extracellular matrix components, remodeling enzymes, cellular adhesion molecules, and growth factors, cytokines, and chemokines genes. The molecular mechanism underlying the healing process still remains unclear. The host response to irritant follows a cascade of events involved in wound healing including vascular and cellular inflammatory events, cellular migration, proliferation and differentiation, angiogenesis and epithelialization, fibroplasia, matrix deposition and remodeling [4]. This process may be enhanced by the production of increased level of free radicals during the inflammatory phase, which leads to the inhibition of cell migration and proliferation, and thus damage the wound nearby tissue. Although the reactive oxygen species activities of healthy and diseased periodontal soft tissues have been documented [5-7], their activities during periodontal wound healing still need to be investigated. Hence, this review aims to highlight the new target and recent mechanisms involved in wound healing.

Tissue injury

The healing process is thought to begin immediately after tissue injury, but multiple systemic and local factors might disturb the natural course of healing, resulting in a chronic,

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non-healing lesion [9], as observed in periodontitis. It is well-understood that an infected root canal system, which acts as a reservoir for microbial cells, virulence products, and antigens that collectively evoke and maintain apical periodontitis [8]. The genes activated during healing events, extracellular matrix (ECM) components, remodeling enzymes, cellular adhesion molecules, growth factors, cytokines, and chemokines are the important factors in the wound healing cascade. The same inflammatory cytokines that promote wound healing can also trigger tissue destruction [10]. In this context, the nature, extent, and duration of the host response seem to play a major role in the determination of healing versus destructive process, and a complex signaling network operates in the determination of lesion outcome. Garlet et al. [11] hypothesized that an infection might result in an imbalance in the expression of wound healing genes involved in periapical lesion pathogenesis. They also believed that the differential expression of wound healing markers in active and/or stable granulomas could account for different clinical outcomes for these lesions.

Wound healing process

Wound healing is a dynamic process, involving phases of inflammation, characterized by vasoconstriction and platelet aggregation to induce blood clotting, proliferation and remodeling that overlap in space and time. Wound healing process includes the following – coagulation, inflammation, proliferation and remodelling. Each phase is predominated by particular cell types, cytokines and chemokines. The inflammatory phase that comprises of increased vascular hyper permeability, damage of skin constituent cells, operation of innate immunity, infiltration of neutrophils and macrophages. Various proinflammatory cytokines interleukin (IL)-1β and pro-IL-18 to IL-1β and IL-18, IL-1α, IL-33, and chemokines HMGB1, and fibronectin are thus produced in the wound tissue at the initial step. The proliferation phase includes angiogenesis, fibroplasia, and re-epithelialization processes and finally remodelling occurs.

Early wound closure can reduce the infection risk, patient morbidity and prevent blood loss from the wound site. Evidence shows that, many factors can disrupt the wound-healing process, including necrotic tissue, chronic diseases, interference with the blood supply, lymphatic blockage, bacterial infection and advanced age. Disruption in the normal healing process leads to the development of chronic wound, which is more difficult to treat and has a higher risk of hypertrophic scar formation upon healing. The initial events are stimulated by an inflammatory response to injury that leads to the recruitment of mesenchymal cells and platelets aggregation at the site of injury with haemostasis, following local vasoconstriction and activation of the clotting cascade, resulting in fibrin clot formation [10]. Mesenchymal cells proliferate and differentiate into chondrogenic or osteogenic lineages in response to bone morphogenetic proteins. During the proliferative phase of wound healing, fibroblasts produce a variety of substances necessary for wound repair, including glycosaminoglycans and collagen.

The periosteum, soft tissue, and bone marrow spaces are potential sources of cells that undergo differentiation. These cells differentiate into chondrocytes that produce cartilage, which helps to mechanically stabilize the fracture callus. After cartilage matrix is formed and mineralized, osteoclasts are generated that cause the resorption of cartilage. During this process, angiogenesis occurs and osteoblasts are formed, leading to subsequent transition from cartilage to bone. The primary bonec is formed, remodeled and reshaped. Interestingly, inflammatory cytokines play an important role in the initial phase of bone repair and later when cartilage is degraded [11]. Among the factors implicated in the control of the wound healing process, an important partner is the extracellular matrix. Fibrin is rapidly degraded by plasmin and neutrophil elastase. This degradation may induce the release of plasma growth factors trapped in the fibrin lattice, which might play an important role in the early events of wound healing [12]. Pro-osteoclastogenic factors, such as tumor necrosis factor (TNF)-α, macrophage colony stimulating factor (MCSF), and Receptor Activator of nuclear factor kappa- B ligand (RANKL), peak during cartilage resorption and are

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particularly important during the transition from cartilage to bone. The importance of TNF-α in these events can be noted from studies involving the absence of TNF-α receptor signaling where there is significantly reduced expression of pro-restorative cytokines, which leads to a delay in the removal of cartilage [13].

Reactive oxygen species (ROS) such as superoxide anions, singlet oxygen, hydroxyl radicals and hydrogen peroxide are generated continuously in aerobic organisms via normal cellular metabolism. At higher concentrations, ROS have harmful and destructive effects on cell membranes, phospholipids, organelles such as lysosomes and mitochondria, DNA and nucleotides as well as promoting degradation of hyaluronic acid, collagen, enzymes and inhibition of cell migration and proliferation. Fibroblasts play a crucial role in wound healing by acting to degrade fibrin clot, create ECM and collagen structures to support the other cells associated with effective wound healing and contract the wound [14]. Therefore, scavenging or detoxification of ROS is crucial for the maintenance of homeostasis in normal tissue and organ systems.

The defence mechanism against ROS includes three anti-oxidant pathways, i.e., intracellular, extracellular and membrane anti-oxidants. The primary system, with this regard, is intracellular ROS scavenging enzymes (FRSEs): superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX). SOD reduces the highly reactive oxygen to less reactive H_2O_2 and it is detoxified to water by CAT and GPX. GPX is also essential for lipid peroxidation and scavenging of OH. The secondary system of detoxification is controlled by extracellular or membranous anti-oxidant compounds such as Vitamins A, C and E, glutathione, NADPH and urate [15]. Detoxification of ROS may be considered to be an essential stage of the normal wound healing process.

Influence of periodontitis on wound healing

Periodontitis is a localized inflammatory response caused by bacterial infection of a periodontal pocket associated with subgingival plaque [16]. Although bacteria are the primary cause of periodontal disease, the expression of microbial pathogenic factors alone may not be sufficient to cause periodontitis. Periodontal pathogens produce harmful by-products and enzymes that break extracellular matrices as well as host cell membranes to produce nutrients for their growth. It initiates damage directly or indirectly by triggering host-mediated responses that lead to self-injury [17].

This periodontal pocket provides ideal conditions for the proliferation of microorganisms: primarily Gram negative, facultative anaerobic species. Prominent amongst these are Bacteroides spp.: B. intermedius and B. gingivalis; Fusiform organisms: Actinobacillus actinomycetemcomitans, Wolinella recta and Eikenella spp.; and various bacilli and cocci; spirochetes; amoebas and trichomonads. The application of growth factors that induce the proliferation, migration, and differentiation of fibroblasts would increase the rate and degree of granulation tissue formation and thus stimulate wound healing [18]. The periodontal pocket, however, remains and if it continues to harbour the bacteria associated with the disease, a potential for a further destructive phase exists.

Delayed healing and formation of chronic wounds have been linked to the excessive production of proteolytic enzymes, leading to reduced amounts of growth factors and successive destruction of the extra cellular matrix (ECM). Extracellular matrix is made of collagen and elastic fibers dispersed in a ground substance made of glycosaminoglycans, proteoglycans and connective tissue glycoproteins. Many data have shown that ECM is able to modulate wound repair, either directly by modulating important aspects of cell behaviour such as adhesion, migration, proliferation or survival, or indirectly by modulating extracellular protease secretion, activation and activity, or modulating growth factor activity or bioavailability [19]. Tissue inhibitor of matrix metalloproteinase 1 (TIMP-1), a member of the TIMP family, has been described as a multifunctional molecule with pleiotropic functions, with roles varying from wound healing

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and regeneration to a wide range of inflammatory and pathologic processes [20]. Matrix metalloproteinases (MMPs) form a multi-gene family within the metalloproteinase class of endopeptidases that collectively mediate the degradation of all ECM molecules [21]. Endothelial cell proliferation accompanies fibroblast proliferation early after injury and neovascularization of the injury region promotes wound healing and repair, while excessive neovascularization beyond the region of injury can promote hemorr hage. The sequestration/release of growth factors by the ECM may prolongate growth factor action or modulate their activity on the cells implicated in the wound healing process

Increased complication rates have been attributed to the presence of teeth in the fracture line. It has been hypothesized that prophylactic tooth extraction can help reduce complications such as infection or non-union. Although the use of antibiotics might be beneficial, the type, route of administration, dosage and duration of antibiotic treatment is still under debate. The Toll-like receptors efficiently recognize distinct components of pathogens that are essential to their metabolism, preventing mutations rendering them undetectable [22]. Other factors, such as oral hygiene, adequate reduction and fixation, and timing of fixation, may be equally or more important than the use of antibiotics. Many authors report that tooth preservation is beneficial for the healing process [23]. According to Zanakis et al. that tooth presence in the fracture line was not associated with healing complications and the rate of complications was higher in fractures with teeth in the fracture line [24]. This decrease in the complication rates might be attributed due to osteosynthesis and the use of antibiotics.

Pulp necrosis of an immature tooth poses potential complications and challenges for treatment. Although treatment techniques for either stimulating formation of a hard tissue barrier or using materials to create an artificial barrier have shown success [25], the long-term effects on the periodontal tissues and tooth structure are still not well- documented. Even if these treatments are successfully performed, the remaining thin dentinal wall makes the tooth weak and increases the risk of subsequent wound healing. An ideal treatment approach of immature teeth with necrotic pulps and apical periodontitis would be to induce an endogenous mineralized structure within the canal space with the aid of surrounding tissues and cells to strengthen these teeth.

In the recent study by Yamauchi et al. [26], the use of a cross linked collagen scaffold significantly increased formation of mineralized tissues. The double-blinded radiographic examination revealed that the occurrence of periapical bone healing and root thickening are both significantly greater in the groups with collagen scaffold in comparison to those without it. Improved healing of the apical periodontitis in the collagen scaffold group was an interesting finding. It might have to do with the osseoinductive properties of the scaffold. It is possible that some of the scaffold was pushed out into the granulation tissue/ periapical tissues during treatment and might have affected healing response, but this needs to be investigated further. Furthermore, in the current study, the effect of dentin matrix exposure with 17% EDTA on the mineralized tissue formation was examined and it was observed that the effect of dentin matrix exposure did not facilitate to form odontoblast-like cells and thus dentin-like structure. The reasons for this might be that firstly, the matrix component was not sufficiently exposed, secondly, the factors important for odontoblast differentiation and mineralization could be extracted with EDTA treatment and thirdly, the mesenchymal stem cells could be more prone to differentiate into cementoblast/osteoblast-like cells when in contact with dentin matrix.

Wound healing and Cytokines

The action of cytokines and growth factors act as promoters of the wound healing cascade by mediating the selective migration and subsequent activation of leukocyte subsets, endothelial cells, and fibroblasts. The periodontal pathogens and inflammatory process is regulated by a sequential network of cytokines which are essential for most periodontal tissue breakdown, leading to clinical signs of disease. The cytokine network takes control over inflammatory mechanisms in order to amplify or suppress tissue

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reactions in periodontal pathogenesis. Prolonged inflammation might impair the healing of chronic wounds via the adverse action of cytokines affecting growth and viability of cells and impeding the integrity of the extra cellular matrix. Mirza et al. [27] in his study, isolated cells directly from wounds and measured cytokine release from these cells ex vivo and established the relative contributions of different cell subsets to the production of cytokines and growth factors during normal and impaired wound healing. The data of the present study provided novel insights into the relative amounts of pro- and anti-inflammatory cytokines and growth factors secreted by different cellular sources during normal and impaired wound healing. Within a persistent infection scenario, chronic periapical lesions might also experience a reagudization process that is correlated with increased leukocyte infiltration; predominance of neutrophils attracted by a chemokine reaction, as well as increased presence of interleukin-17 [28]. Recently, the expression of the midkine (MK) gene by inflammatory cells such as macrophages, lymphocytes, and neutrophils has also been reported as an important factor during development of periapical granulomas [29]. The various interleukin family of cytokine types are given in Table 1.

Platelet-rich plasma (PRP) is a portion of blood plasma with platelet concentration greater than baseline [30]. It is a rich source of cytokines, chemokines, growth factors and matrix proteins [31,32]. Application of certain growth factors, such as platelet-derived growth factor, vascular endothelial growth factor, transforming growth factor-β and epidermal growth factor, have been shown to exert beneficial effects on healing [33]. PRP has normally been applied clinically as platelet gel, with the addition of calcium chloride and/or thrombin to stimulate the polymerization of fibrinogen content within the PRP. Keratinocyte and fibroblast proliferation is critical in the wound-healing process. Differentiated keratinocytes lose the ability to self-renew and proliferate, limiting their role in wound healing [34]. Following injury, keratinocytes become mobilized by undergoing phenotypic changes favouring detachment in a process that remains incompletely understood. However cytokines such as IL-1, IL-6 and TNF-α produced in inflammatory phase seem to help modulate the migratory phenotype of keratinocytes [35].

Accumulation of matrix proteins at injured sites is fundamentally important step in granulation tissue formation to support the migration and proliferation of cells. Keratinocytes and fibroblasts responded oppositely to PRP in term of migration: the keratinocyte migration was mildly increased, and the fibroblast migration was significantly slower. Reduction in fibroblast migration might be due to the presence of cystine, which inhibits fibrin a-chain cross-linking [36]. Keratinocytes and fibroblasts secrete a variety of soluble factors, including cytokines, chemokines and growth factors that regulate wound healing [37].

Quantitative analysis with multiplex ELISA revealed that the 20% PRP culture contained significantly higher granulocyte-macrophage colony-stimulating factor, a potent neutrophil and monocyte/ macrophage chemo attractant that also promotes reepithelialization and angiogenesis. IL-1α, which was 7-fold higher in the 20% PRP group (but not significantly different), is a potent inflammatory mediator [38]. Reducing the PRP concentration by half stimulated the cells to secrete significantly higher hepatocyte growth factor, monocyte chemo attractant protein-1, epithelial-derived neutrophil-activating protein78 and vascular endothelial growth factor A, as well as IL-6, which play requisite roles in granulation tissue formation, leukocyte chemotaxis, angiogenesis, re-epithelialization and tissue remodeling. These results suggest that higher PRP concentration favors inflammation, while lowering the concentration shift the healing process from inflammatory phase to proliferation and remodeling phases [39].

The keratinocyte and fibroblast wound-healing enhances inflammation and collagen deposition. In the future, acute and chronic wounds which have different wound physiology and healing dynamic, consideration should be given to the optimal PRP concentration to treat each type of wound to promote healing, and also to improve the appearance of scars after healing. Impairment of wound healing occurs as a consequence of

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ischemia, excessive reactive oxygen species (ROS), and inflammatory cytokine production [40]. The repair of periodontal wounds is considered a complex process because of the particularities of the medium where they are located. Besides masticator trauma on the wound site, the oral cavity is filled with microorganisms, which makes it one of the most complex and dynamic medium of the body involved in numerous physiologic processes, and this may alter the normal healing process.

In view of the above facts Mahendra et al. [41], hypothesized that topically applied deferoxamine could also accelerate cutaneous wound healing in diabetic rats by modulating some important markers of wound healing, such as, hypoxia-inducible factor 1-alpha (HIF-1α), vascular endothelial growth factor (VEGF), stromal cell-derived factor1-alpha (SDF-1α), transforming growth factor beta1 (TGF- β1), tumor necrosis factor-alpha (TNF-α), matrix metalloproteinase-9 (MMP-9), interleukin-1 beta (IL-1β) and Interleukin-10 (IL-10), matrix synthesis, inflammatory cells, etc. which abnormally behave in wounds.

Inflammatory cytokine gene expression is a process strictly regulated by various mechanisms, including the negative regulation of intracellular signaling. Endogenous proteins are involved in this process, but the mechanisms by which these proteins regulate gene expression are still elusive, especially in periodontal disease. The Suppressor of Cytokine Signaling (SOCS) family of proteins modulates in a fairly specific manner the JAK/STAT pathway, which is critical in signal transduction in inflammation. In the recent study [42] it was observed that the kinetics of SOCS1 and SOCS3 expression was directly correlated with the severity of inflammation, alveolar bone resorption and the level of pro-inflammatory cytokine expression during the course of experimental periodontal disease. This suggests that these proteins play an important role in the modulation of host response. Understanding the role of SOCS proteins in the negative regulation of cytokine signaling may provide novel information on the susceptibility to periodontal diseases and also for therapeutic strategies based on the modulation of the inflammatory process.

Conclusion

The various factors affecting and accelerating wound healing is rapidly in progress. There is an imbalance in the expression of wound healing genes involved in the pathogenesis of periodontal lesions. This review would summarize main findings showing the effects of cytokines on periodontal wound healing process. Every cell type involved in wound healing, inflammatory cells, keratinocytes, fibroblasts, endothelial cells or pluripotent stem cells are concerned by these interactions. Since wound healing is a well-concerted process with numerous factors, a simple activation or inhibition of a single factor might affect favorable therapeutic efficacy. To better understand wound healing, the molecular mechanisms involved in periodontal wound healing matrix interactions, inflammatory-immune response and tissue destructive mechanisms, analysis of individual molecules/genes may be required to constitute new targets for therapeutic strategies in the management of the wound healing defects in periodontitis.

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