Review Article

ROLE OF GROWTH FACTORS (TGF-β, PDGF, KGF, FGF, Pro Collagen, VEGF) IN WOUND HEALING

ABSTRACT

The wound healing process is an intricate biological phenomenon that requires various crucial components, including repair cells, proteins, and biological factors. The process of wound healing can be categorized into three interrelated stages: the stage of inflammation, the stage of proliferation, and the stage of remodeling. Any interruptions that arise throughout this process can lead to wound healing that deviates from the typical progression. It is crucial to highlight that growth factors have a profoundly influential effect in this particular situation. This study conducts a thorough and analytical literature review to investigate the essential role of growth factors (TGF-β, PDGF, KGF, FGF, Pro Collagen, and VEGF) in the process of wound healing. This study examines the biochemical and molecular mechanisms via which these growth factors impact the wound healing process, including the inflammation, proliferation, and remodeling phases, by analyzing current and authoritative scientific literature. This paper precisely outlines the role of these variables, including the role of the stem cell secretome enriched with growth factors such as TGF-β, PDGF, KGF, FGF, Pro Collagen, VEGF, and exogenous factors, as previously discussed. It explores their impact on tissue regeneration, angiogenesis, and extracellular matrix formation, essential components of the wound healing process. The main objective of this study is to provide a comprehensive summary of the latest research findings, which includes an in-depth analysis of the stem cell secretome's contributions to wound healing. The findings provide useful insights into potential growth factor-based treatment approaches, highlighting how the components of the stem cell secretome can be leveraged to enhance the wound healing process.

Keywords: Wound Healing, Growth Factors, tissue regeneration, angiogenesis, extracellular matrix formation

1. INTRODUCTION

Over millennia, human skin has undergone extensive evolution, resulting in the development of a specialized organ that effectively shields the body from harm caused by chemical, physical, and UV radiation. Unavoidably, contact with the outside surroundings leads to damage to the skin. Hence, it is unsurprising that our skin possesses specialized mechanisms to mend injured tissue, enabling it to recuperate promptly and effectively. A wound is defined as any damage to tissue that causes a disruption in its structure and function. The wound healing process primarily involves the restoration of damaged skin tissue. The process of wound healing commences immediately upon the injury of the epidermis and might persist for a span of multiple years. This dynamic process encompasses highly coordinated mechanisms occurring at the cellular, fluid-based, and

molecular levels.[1,2] The process of wound healing can be categorized into three interconnected phases: inflammation, proliferation, and remodeling. Any interruption that takes place during this phase can lead to atypical wound healing. An organism's capacity to repair or regenerate tissue is a notable benefit for its survival. Abnormal wound healing occurs when the natural course is disrupted. Wounds pose a significant worldwide health concern. In nations like the UK and Denmark, the prevalence of persons with one or more lesions is estimated to be around 3 to 4 per 1000 people. A significant number of these injuries develop into long-lasting conditions, and regrettably, 15% of them persist without healing even after a year of medical intervention. Aside from the physical, psychological, and social consequences, the loss of workplace productivity, coupled with costly medical interventions for wound care, imposes a financial strain on the healthcare system.[2,3]

Wound healing is an intricate biological phenomenon that encompasses various components, including reparative cells, proteins, and biological stimuli. Of these, growth variables have a significant impact. These growth factors promote the growth, differentiation, and movement of repair cells such as keratinocytes, fibroblasts, and vascular endothelial cells (VECs). Furthermore, growth factors govern other facets including the programmed cell death (apoptosis) of repair cells, the composition of the extracellular matrix (ECM), the creation of DNA, RNA, and proteins, glycolysis, and the restoration of damaged tissue. Speculation exists regarding a potential decrease in the activity and/or quantity of growth factors and their related receptors as a possible underlying cause of non-healing wounds. This notion establishes a theoretical foundation for the localized administration of epidermal growth factors (eGFs) to promote the process of wound healing in different situations.[4,5]

Wound healing can be classified into two categories: primary healing and secondary healing. Primary healing denotes the prompt and complete recuperation of a well protected, uninfected lesion. Wounds resulting from surgical procedures exemplify initial healing. Nevertheless, if the process of wound healing is interrupted by factors such as infection, wound reopening, inadequate oxygenation, or immune system abnormalities, a subsequent phase of healing will ensue. Secondary healing occurs when granulation tissue forms and is subsequently covered by epithelial cells. These wounds have a higher vulnerability to infection and typically exhibit suboptimal healing outcomes. The treatment provided typically focuses on preventing and accelerating the rate of delayed wound healing associated with this condition. Hence, acquiring a more profound comprehension of the physiological mechanisms involved in wound healing can foster the pursuit of additional investigations aimed at enhancing the efficiency and efficacy of wound care.[6,7]

The wound-healing process is significantly influenced by the secretome, a complex mixture of substances secreted by stem cells. This secretome is rich in various growth factors, including exogenous growth factors, Fibroblast Growth Factor (FGF), as well as Transforming Growth Factor-beta (TGF-β), Platelet-Derived Growth Factor (PDGF), Keratinocyte Growth Factor (KGF), Fibroblast Growth Factors (FGF), Pro Collagen, and Vascular Endothelial Growth Factor (VEGF). These elements are crucial in accelerating the wound-healing process by enhancing existing skin cells' proliferative and migratory capabilities. The stimulation of skin cells occurs through multiple signalling pathways, each contributing to tissue repair and regeneration. These diverse growth factors, particularly exogenous factors along with TGF-β, PDGF, KGF, FGF, Pro Collagen, and VEGF, highlight the potential of secretome therapies. These therapies enhance the body's natural healing processes, offering promising avenues for advanced wound care treatments by facilitating cell division, movement, and angiogenesis, which are essential for effective wound healing.Treatment modalities available for wounds include surgical and non-surgical interventions. Exogenous growth factors (eGFs) are a significant non-surgical approach to enhance wound healing efficiency and provide a favorable wound healing environment.

Exogenous growth factors (eGFs) have achieved widespread acceptance and have been implemented in numerous nations for approximately thirty years. Commercially accessible eGFs were first used successfully in wound care thirty years ago. Since then, there have been no notable instances of toxicity or serious adverse responses linked to the use of eGFs for wound care.[4,8] Neverthelessthe role of epidermal growth factor (eGF) has garnered significant interest. However, its efficacy in this context remains a topic of ongoing debate among medical professionals and researchers. Therefore, this study has been conducted to examine the role of eGF in the wound healing process, aiming to provide clearer insights and evidence-based conclusions about its effectiveness.

2. MATERIAL AND METHODS

This study is a literature review that aims to find database sources and primary information sources, including Scopus, EMBASE, PubMed, and Google Scholar. The selection of this database was based on its comprehensive compilation of peer-reviewed scholarly papers. Chosen publications must satisfy specific requirements, including being published within the past decade. However, if there is crucial information with a reference older than 10 years, additional evaluation will be conducted to ensure comprehensive information. This research specifically investigates the function of growth factors in the process of wound healing. The research material must consist of original research articles, reviews, or meta-analyses.

The keywords and search phrases will be organized in a way that captures pertinent literature. The following keywords will be utilized in various combinations: "TGFβ", "PDGF", "KGF", "FGF", "Pro Collagen", "VEGF", and "wound healing". Following the initial search, a process of filtering will be conducted using the title and abstract to assess the article's pertinence to the topic. Articles that satisfy the above criteria will be further examined for a comprehensive analysis. The gathered data will undergo analysis to identify prominent themes, patterns, and conclusions within the literature. The focus will be on the specific involvement of each growth factor in the process of wound healing. Information from multiple sources will be combined to create a logical and thorough account of the topic being investigated.

3. RESULTS AND DISCUSSION

WOUND HEALING MECHANISMS

The process of wound healing has three interconnected stages: inflammation, proliferation, and remodeling, which occur in a sequential manner.[9]

Inflammation

This stage encompasses the processes of hemostasis and inflammation. Upon skin injury, the body promptly triggers its hemostatic process, which focuses on generating a fibrin blood clot to seal the wound at the site of injury. Simultaneously, the affected region undergoes vasoconstriction, a process that narrows the blood vessels, for a duration of about 5 to 10 minutes. This mechanism serves to halt bleeding and safeguard the wound. Furthermore, the formation of the fibrin plug acts as a provisional scaffold for the wound, aiding in the future healing process by promoting the movement of different cell types, such as leukocytes, keratinocytes, fibroblasts, and endothelial cells. This creation also serves as a repository of growth factors. Following the initial period of vasoconstriction, there follows a subsequent phase of vasodilation. Vasodilation leads to augmented blood circulation towards the affected region, thereby inducing swelling (edema) in the wounded area. The

alterations in blood circulation and the presence of inflammation are the primary mechanisms via which the inflammatory response to tissue injury manifests.[1,9]

The occurrence of tissue injury stimulates the process of platelet aggregation and initiates the discharge of platelet granules. The granules contain a diverse array of signaling chemicals, such as chemotactic factors and growth factors. Within the initial 24-hour period following an injury, neutrophils are attracted to the site of injury and persist there for a duration of 2 to 5 days. Neutrophils have a crucial function in starting phagocytosis, which is subsequently carried out by macrophages. Phagocytic cells, such as neutrophils and subsequently macrophages, secrete reactive oxygen species (ROS) and proteases. These compounds are designed to counteract the effects of germs in a specific area and remove dead tissue. Neutrophils not only work as phagocytes, but they also serve as chemoattractants for other cells and contribute to the intensification of inflammatory reactions by releasing several pro-inflammatory cytokines. Macrophages, however, reach the site of injury around 3 days following the initial tissue damage. Similar to neutrophils, they also secrete a variety of growth factors, chemokines, and cytokines. These signaling molecules have a crucial function in stimulating cell growth and the production of extracellular matrix (ECM) components, which aids in tissue repair and regeneration.[1,4]

Proliferation

The proliferative phase is characterized by the formation of granulation tissue and restoration of blood vessel tissue. This phase usually begins approximately 3 to 10 days after injury and can take several days to weeks to complete. Cytokines and growth factors play important roles in this phase, including members of the transforming growth factor-beta (TGF-β, including TGF-β1, TGF-β2, and TGF-β3) family, interleukins (IL), and factors that promote angiogenesis. The main cells involved in proliferation are fibroblasts and endothelial cells. The process of cell proliferation requires an adequate blood supply, thus triggering a simultaneous angiogenic response.[1,6]

Local oxygen deficiency, vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), and thrombin are among the stimuli that induce this process of blood vessel formation (angiogenesis). Conversely, epithelialization commences upon damage, stimulated by inflammatory cytokines and other growth factors. This process involves the involvement of keratinocytes located at the borders of the wound, as well as epithelial stem cells found in the bulbs of hair follicles and apocrine glands. The stem cells undergo differentiation into keratinocytes, which subsequently migrate across the wound until they come into physical touch with one another. At this stage, migration ceases due to contact resistance. The last stage of the proliferation phase entails the development of granulation tissue. Fibroblasts travel towards the wound site and undergo development within it. The fibroblasts initiate the production of a temporary matrix that consists of type III collagen, glycosaminoglycans, and fibronectin. Granulation tissue is composed of fibroblasts, granulocytes, macrophages, capillaries, and loosely organized collagen tissue.[4,6]

Remodeling

The remodeling phase of wound healing, typically commencing around on day 21 and potentially extending for up to a year, signifies the concluding stage of this procedure. During this phase, there is a synchronized process of both tissue creation and degradation, which happens continually. Any disruption to this process might lead to the development of chronic wounds. During this stage, the process of granulation is finished and the wound enters the maturation phase. The extracellular matrix (ECM) undergoes specific alterations to enhance

its strength and organization. Type III collagen, which is less resilient, is substituted with type I collagen, which is more robust. Over time, the elasticity gradually strengthens. The process of collagen synthesis persists for around 4 to 5 weeks. Nevertheless, the collagen present in the wound region never attains the equivalent degree of flexibility observed in intact skin. Collagen synthesis necessitates the presence of oxygen and vitamin C. Therefore, both hypoxia and vitamin C deficiency can potentially impact wound strength.[1,4]

Enzymes, particularly MMPs, have a significant impact on altering the conditions of the matrix in specific regions. They also influence processes such as cell migration, proliferation, and angiogenesis. Cells from earlier stages undergo programmed cell death, known as apoptosis, while alterations in the wound's characteristics take place. TGF-β1 induces fibroblasts to undergo differentiation into myofibroblasts. These myofibroblasts play a role in producing essential extracellular matrix (ECM) proteins, including collagen types I to VI and XVIII, glycoproteins, and proteoglycans. Additionally, they are involved in wound contraction. Myofibroblasts in this state have similar traits to smooth muscle cells, since they express alpha smooth muscle actin and exert robust contraction forces to bring together the wound margins, so assisting in wound closure. Upon full epithelialization of the wound, myofibroblasts undergo apoptosis. Hyperactive myofibroblasts can result in fibrosis and the development of scars. The apoptosis of fibroblastic cells plays a crucial role in the development of fully formed wounds, which are characterized by a relatively high number of cells. The angiogenic response will diminish when the repair process concludes, blood flow decreases, and acute metabolic activity in the wound stops. This concurrent process results in the closure of the damaged tissue and the restoration of its mechanical integrity. Scar development is the ultimate outcome of wound healing. Nevertheless, this utilized tissue possesses specific constraints, such as its inability to attain the equivalent level of resilience as healthy skin. After a period of three months, the wound had only regained around 80% of its initial strength. Furthermore, subepidermal tissues, such as hair follicles and sweat glands, do not undergo regeneration following significant injury. Furthermore, the absence of rete pegs, a component responsible for the robust attachment between the epidermis and dermis, can be observed in the aforementioned tissue.[4,9]

WOUND MANAGEMENT

The basic objectives of wound care management are to achieve two primary goals: rapid wound closure and optimal wound healing (functional and aesthetic). While the fundamental principles for obtaining optimal wound healing may appear straightforward, the process of wound healing is not always devoid of obstacles. Frequently, a multitude of difficulties may occur throughout the course of wound healing. Hence, it is crucial to meticulously uphold optimal circumstances that promote the innate and physiological progression of wound healing, with particular emphasis on sustaining the requisite moisture levels within the wound.[8,10]

For over a decade, the process of preparing the wound site to promote the regrowth of skin on chronic wounds has been a crucial part of managing wound care. Wound debridement is founded on the concept that it can promote re-epithelialization, and this strategy has been a fundamental component of wound care administration for more than a decade. The process entails analyzing the indications, contraindications, and strategies related to wound debridement, with a focus on the collaborative role of the healthcare team in the treatment of patients undergoing this surgery. Debridement is crucial in the treatment of wound care as it prepares the wound site for re-epithelialization. Bacteria can obtain nourishment from dead tissue, which includes necrotic tissue. In addition, deceased tissue serves as a physical obstruction, impeding topical substances from directly contacting the wound area and delivering their advantageous characteristics. Necrotic tissue hinders processes such as the

generation of new blood vessels (angiogenesis), the formation of granulation tissue, the closure of the outermost layer of skin (epidermis), and the normal development of the network of molecules that support cells (extracellular matrix or ECM). In essence, the existence of necrotic tissue has the ability to obscure the true scope and seriousness of the damage, so concealing a possible underlying infection.[3,9]

It is necessary to thoroughly evaluate every wound in terms of its status. This assessment entails a meticulous analysis of multiple factors, such as the dimensions of the wound, its level of penetration, and the existence of foreign bodies or infections. Thorough wound cleaning is crucial as it helps eliminate any foreign objects, necrotic tissue, or harmful microorganisms that may impede the wound's healing. Moreover, it is crucial to make careful adjustments in the selection of treatment methods and materials to correspond appropriately with the particular stage of wound healing that the patient is undergoing, whether it is the initial inflammatory stage, the proliferative stage marked by tissue regeneration, or the late stage characterized by tissue remodeling. Wound care management is a complex field that focuses on maximizing healing outcomes. This requires a methodical evaluation, suitable intervention, and a comprehensive understanding of the factors that influence the wound healing process.[11,12]

THE ROLE OF GROWTH FACTORS IN WOUND HEALING

Wound healing is an intricate biological process that depends on intricate connections between many cellular and molecular systems. Growth factors play a pivotal role in orchestrating the interactions between cells and the surrounding matrix in the innate wound healing process. These growth factors work as signaling molecules that control the activity of many cell types involved in the process of wound healing, including keratinocytes, fibroblasts, and vascular endothelial cells (VECs).[8,13]

The absence of appropriate growth factors, especially due to reduced production and high levels of breakdown of these factors, is a triggering factor for chronic wounds. These non-healing wounds are often caused by a variety of underlying medical conditions, including diabetes, vascular disease, and immune system disorders. The presence of reduced growth factors results in an inhibition of the normal progression of wound healing, resulting in delayed or incomplete tissue repair that occurs. To overcome this challenge, researchers and clinicians have explored the potential of introducing external growth factors into non-healing wounds. The idea is to supplement the deficient local supply of these important molecules, in the hope of improving cellular response and promoting timely wound closure.[11,12]

Transforming Growth Factorβ

TGFβ is a diverse collection of cytokines comprising three variations: TGFβ1, TGFβ2, and TGFβ3. TGFβ1 is the primary factor in skin wound healing among the three. The distribution of these growth factors differs among various strata of the epidermis. TGFβ1 is mostly present in the stratum granulosum and corneum, while TGFβ2 and TGFβ3 are detected in layers above the basal layer, suggesting distinct functions. TGFβ is originally produced as a bigger pro-peptide, which is in an inactive latent state. The N-terminal latent-associated peptide is attached to the C-terminal mature TGFβ in a noncovalent manner in this arrangement. The activation of this dormant state can be triggered by proteases, integrins, thrombospondin-1, reactive oxygen species, low pH, heat, and mechanical stresses. This stimulation results in the secretion of fully developed and functionally effective growth factors.[14,15]

TGFβ is crucial for both the homeostasis of epidermal tissue and the various phases of wound healing. This engagement encompasses the control of diverse cell types, including keratinocytes, fibroblasts, endothelial cells, monocytes, and others. TGFβ1 is the most extensively researched growth factor in wound healing, as it has significant and widespread impacts on keratinocyte migration. All three isoforms of TGFβ play a role in the processes of wound healing and re-epithelialization. TGFβ1 expression significantly and promptly rises following acute tissue injury, and is released by various cell types such as keratinocytes, platelets, monocytes, macrophages, and fibroblasts. TGFβ1 has a crucial function in initiating the inflammatory response and promoting the production of granulation tissue. Furthermore, it stimulates the process of wound contraction by triggering the expression of smooth muscle alpha actin in fibroblasts and facilitating the development of myofibroblasts. Furthermore, TGFβ1 contributes to angiogenesis by elevating levels of vascular endothelial growth factor. Finally, TGFβ1 promotes the movement of keratinocytes, which is crucial for the process of wound healing.[14,15]

Platelet-derived Growth Factor

PDGF displays a wide range of biological functions through its interaction with target cell membrane receptors. These interactions initiate a cascade of biological responses that are very pertinent to both normal and pathological tissue healing processes. These effects involve attracting inflammatory and repair cells towards the wound through chemotaxis, promoting the proliferation of different cell types such as vascular endothelial cells (VECs), fibroblasts, smooth muscle cells (SMCs), and epithelial cells, aiding in the creation and rearrangement of the extracellular matrix (ECM) to facilitate the regeneration of blood vessels, assisting in the formation of granulation tissue, and facilitating the restoration of the epithelial layer at the wound site.[16,17]

Platelet-derived growth factor (PDGF) holds the unique distinction of being the initial growth factor linked to wound healing that has been thoroughly examined and separated. This growth factor is a highly significant and potent stimulant that plays an active role in nearly every stage of the wound healing process. Platelets are primarily responsible for initiating the release of PDGF after degranulation. Additionally, PDGF is secreted by keratinocytes, fibroblasts, endothelial cells, and macrophages. PDGF has a multifunctional purpose that involves serving as a mitogen, which means it stimulates the proliferation of different cell types like fibroblasts, keratinocytes, and endothelial cells. Furthermore, PDGF stimulates the targeted movement of neutrophils, macrophages, fibroblasts, and smooth muscle cells towards the site of injury. This action is crucial for initiating the inflammatory phase. PDGF acts as a chemoattractant for bone marrow mesenchymal stem cells, which gather at the wound site and help create fibroblasts. PDGF stimulates fibroblast proliferation and enhances the production of crucial components of the extracellular matrix (ECM), such as fibronectin, collagen, proteoglycans, and hyaluronic acid.[17,18]

Keratinocyte Growth Factor

The protein KGF has a molecular weight of around 26-28 kDa and is present as a single molecule. Approximately 66% of its carboxyl-terminated cDNA has a resemblance of around 30-45% to eight additional proteins belonging to the FGF family. These growth factors are synthesized by many stromal cell types in multiple organs including the lungs, prostate, mammary glands, stomach, bladder, and skin. Unlike epithelial cells, KGF is not synthesized by the epithelial cells themselves. Instead, it functions as a mediator that is particular to the epithelium, predominantly through paracrine mechanisms. The importance of KGF lies in its role in tissue growth and its ability to regulate the healing process of the skin. Although it is not commonly found in healthy human skin, its expression in dermal fibroblasts (DFB)

considerably increases following skin injury. Keratinocyte Growth Factor (KGF) stimulates the movement and multiplication of keratinocytes by interacting with their cell membrane, causing them to migrate from the wound's periphery into its core. This action promotes the process of re-epithelialization of the skin.[19,20]

KGF acts as a catalyst, stimulating the migration and proliferation of keratinocytes (KC), thereby playing a crucial role in fostering the regeneration of the skin's outer layer. Upon injury, dermal fibroblasts (DFB) commence the synthesis of KGF, which subsequently attaches to the KC membrane, specifically targeting the FGFR2 receptor, a variant of receptor tyrosine kinase. This receptor is highly expressed in keratinocytes (KCs) in the basal layer of the skin and in hair follicles. However, its expression is noticeably missing in dermal fibroblasts (DFBs), highlighting its role in mediating connections between mesenchymal and epithelial cells through paracrine signaling. In a broader sense, the attachment of these growth factors to receptors initiates pathways that control many elements of cellular function and subcellular biology. To be more precise, the binding of KGF to its receptor triggers the addition of phosphate groups to tyrosine residues on the receptor. This causes the ligand-receptor complex to come together and form a structure called a clathrin-capped pit, which is then taken into the cell by a process called endocytosis. This intricate entity traverses both the first and final stages of the endocytic pathways, ultimately reaching perinuclear structures and subsequently being conveyed to the compartment responsible for lysosomal breakdown. Although the degradation process of the receptor occurs gradually, it continues to function in late endosomes. In this location, the receptor is degraded by a particular route as a result of KGF-induced ubiquitination. Within the nucleus of a cell, KGF stimulates the activation of rescue pathways and enhances the production of crucial enzymes involved in the creation of new substances. KGF possesses the capability to initiate the synthesis of crucial nucleotides that are required for the replication of DNA, the synthesis of RNA, and subsequently, the proliferation of KCs throughout the process of wound healing. This process guarantees a sufficient provision of the nucleotides required for its cellular functions. Furthermore, research has demonstrated that KGF not only facilitates cell division, but also enhances the movement of KCs. The metalloproteinase stromelysin-2 may play a role in this action by regulating the movement of KC cells through breaking down proteins that are involved in the adhesion between cells and between cells and the surrounding matrix. The proteolytic activity aids in the liberation and mobility of KCs, which is crucial for the re-epithelialization process.[19,21]

Fibroblast Growth Factor

Fibroblast growth factors (FGFs) are an important category of growth factors found in organisms. Fibroblast growth factors (FGFs) exert a wide range of effects on tissues and cells that originate from the mesoderm and neuroectoderm. The functions of this include the repair of wounds and nerves, control of metabolism, formation of new blood vessels, and development of embryos. Two distinct variants of FGF, specifically bFGF and aFGF, have been extensively utilized. The potential role of bFGF in expediting wound healing encompasses various factors. It primarily induces angiogenesis in both laboratory settings and real beings, attracting diverse types of cells involved in angiogenesis and promoting their proliferation and migration. It plays a substantial role in the process of angiogenesis. After an injury, basic fibroblast growth factor (bFGF) attracts monocytes, neutrophils, macrophages, and fibroblasts to the damaged area through chemotaxis. This process supports the creation of granulation tissue, cell division, and growth, which are essential for tissue repair. Similarly, aFGF induces the activation of fibroblasts and other crucial cells involved in the process of skin restoration, hence enhancing the healing of wounds. It functions by stimulating cell division and proliferation (mitogenic activity) and by decreasing local ischemia (non-mitogenic activity). Therefore, aFGF acts as a stimulant for cell division

in several types of cells, playing an active role in multiple aspects of tissue regeneration.[22,23]

Vascular Endothelial Growth Factor

VEGF, a glycoprotein that consists of two identical subunits, shares around 20% of its amino acid sequence with platelet-derived growth factor (PDGF). VEGF has five isoforms resulting from different mRNA processing events, characterized by variations in chain length (121, 145, 165, 189, and 206 amino acids). The five types are commonly referred to as VEGF-A (VEGF165), VEGF-B, VEGF-D, and placental growth factor (PlGF). Endothelial cells, fibroblasts, smooth muscle cells, platelets, neutrophils, and macrophages, all contribute to the production of VEGF during wound healing. VEGF plays a crucial role in wound healing by primarily promoting angiogenesis, which involves many phases like vasodilation, breakdown of the basement membrane, migration of endothelial cells, and their subsequent proliferation. The process concludes with the development of capillary tubes, the connection of parallel capillary shoots (loop formation), and the creation of a new basement membrane.[24]

VEGF promotes the movement of endothelial cells in wound healing by two primary mechanisms: chemotaxis, which is the directed movement of cells towards a chemical gradient, and vasodilation, which is the widening of blood vessels. During the initial phases of angiogenesis, endothelial cells undergo migration prior to engaging in mitotic division. Capillary sprout growth can persist for a duration of 4 or 5 days via means of endothelial elongation and migration, without involving cell proliferation. VEGF prolongs the lifespan of endothelial cells, overcomes senescence, and reinstates their capacity to proliferate. Additionally, it hinders programmed cell death by temporarily enhancing the production of two anti-apoptotic proteins in human endothelial cells. This has the potential to shield against apoptosis triggered by stimuli like TNF-α and ionizing radiation.[25,26]

Pro Collagen

Collagen, the predominant protein in the body, is synthesized by cells like fibroblasts during the process of wound healing and transformed into intricate structures. Collagen undergoes alterations in its kind, quantity, and organization during the process of wound healing, ultimately determining the tensile strength of the healed skin. During the early stages of wound healing, the production of type III collagen takes place initially. However, this collagen is subsequently replaced by type I collagen, which serves as the predominant collagen in the skin. During the creation of granulation tissue, collagen is first arranged in a disorganized manner. However, it later undergoes covalent cross-linking facilitated by the enzyme lysyl oxidase. This process refines collagen into a complex structure that is prepared to acquire tensile strength. The process of collagen restructuring persists for several months after the wound is closed, resulting in a strengthening of the restored tissue to around 80-85% of its original strength, provided that the process is not interrupted.[5,27]

Collagen is essential for imparting mechanical strength and elasticity to tissues, as well as acting as a natural surface for cell attachment, proliferation, and differentiation. The formation of biofilm triggers the activation of MMP-2 through microRNA, resulting in the degradation of collagen. This process predominantly leads to a decrease in the ratio of collagen I to collagen III. This hinders the biomechanical characteristics of healing skin, which could potentially make it more prone to the reappearance of wounds. Recent analyses of collagen structure and function indicate that in healthy tissue that has been wounded, collagen fibrils assume a closed shape. When blood comes into contact with the injured area, this structure unfolds, exposing specific areas where cells and ligands can attach. This

attachment can speed up the process of healing the wound. Collagen is a crucial component of the extracellular matrix (ECM), playing a role in both skin elasticity and the stabilization of growth hormones. Additionally, it helps regulate cell adhesion and communication between cells and the ECM. Through the process of scar tissue remodeling over a period of time, wounds that have fully developed eventually heal, resulting in the formation of a scar that is considered "normal". The tensile strength of this utilized tissue is approximately 50-80% of that of regular skin, but it may not have complete functionality. The primary distinctions between scarred and unscarred skin are evident in the density, size, and orientation of collagen fibrils.[10,28]

THE ROLE OF THE SECRETOM IN WOUND HEALING

The secretome, derived from mesenchymal stem cells (MSCs), has a crucial function in the process of regeneration by releasing vital molecules that stimulate the repair and rejuvenation of injured keratinocytes and dermal fibroblasts. The secretome releases important growth factors such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and epidermal growth factor (EGF). When the secretome is administered to the region of damaged tissue, it enhances the development of connective tissue components, stimulates the formation of blood vessels, and releases cytokines and growth factors that aid in tissue regeneration and the wound healing process. Prior research has highlighted the regenerating capacity of the secretome and has shown that it acts as a non-tumorigenic, secure, and immunomodulatory agent that may be transplanted. The secretome has exhibited the capacity to repair diverse tissue types, such as liver, heart, bone, cartilage, adipose tissue, pancreas, nerves, blood vessels, and cutaneous components. The secretome releases several proangiogenic and wound healing factors, including transforming growth factor (TGF), VEGF, platelet-derived growth factor, insulin-like growth factor, and interleukin (IL)-6. The management of chronic wounds faces several challenges, such as a healing rate of less than 50% and the limitations and expenses linked to existing treatment methods. With the rapid progress of technology, there is a pressing requirement for inventive strategies in the field of wound care. Secretome therapy is a promising and rising alternative to traditional wound healing methods. It utilizes the intrinsic growth factors found in the secretome to promote healing.[29]

Wound treatments that utilize secretome-based approaches have demonstrated encouraging outcomes by harnessing the regenerative capacity that can induce differentiation into distinct cell types. The versatility of secretome as a feasible therapeutic choice in various illnesses is evidenced by its flexibility, and the lack of significant adverse effects linked to this treatment validates its safety profile. The growing utilization of the secretome in diverse pathologies highlights the versatile characteristics of the secretome and its potential application in numerous medical disciplines. Studies on the bone marrow-derived secretome (BM-Sec) have uncovered its function as a precursor that differentiates into many cell types, including fat, chondrocytes, and dense osteocytes, along the germline pathway. Recent study highlights the therapeutic effects of the secretome in regulating the milieu of tissues and the immune system's ability to respond, therefore speeding up wound healing and decreasing the formation of fibrosis or scars. This therapeutic approach has been widely used in the field of regenerative medicine, specifically to tackle the intricate difficulties associated with chronic wounds. The number is 16. The case study by Sarasua et al demonstrates the effective closure of a type IV decubitus ulcer within 18 days following the injection of BM-Sec.[30]

The therapeutic mechanisms of the secretome involve the stimulation of physiological processes in damaged tissue, as well as the secretion of different growth factors and cytokines derived from the secretome. The secretome derived from stem cells can be

obtained in vitro using a medium known as conditioned media. The secretome is an abundant reservoir of vital growth factors, such as FGF and VEGF, that play a pivotal role in controlling the intricate process of wound healing. Specifically, VEGF plays a crucial role in preserving the oxygenation of tissues that are undergoing hypoxia. The expression levels of FGF and VEGF, which serve as markers of the MSC secretome, were evaluated using flow cytometry. The assessment of Pro-Collagen 1 was conducted by enzyme-linked immunosorbent assay (ELISA), while fibroblast cell viability was determined using the cell counting kit (CCK-8). The secretome engages in paracrine signaling, which allows it to communicate with different cell types such as immune cells, fibroblasts, and endothelial cells. This interaction helps to regulate the process of wound healing. In vitro studies have also revealed the paracrine effects between the secretome, local tissue cells, and different types of immune cells, all of which are controlled by secretory factors produced by the secretome. Administering secretome in vivo has been demonstrated to promote the migration of endothelial cells and macrophages, while decreasing effector T cells. This, in turn, initiates angiogenesis and regenerative processes.[31]

During the initial week of treatment, the secretome exhibited a notable change in the color of the wound bed, transitioning to a pink hue. This finding provides evidence that the first process of secretome administration is mediated by VEGF. VEGF induces the formation of new blood vessels and encourages the growth of existing ones. It enhances the delivery of nutrients and allows inflammatory cells, such as macrophages and neutrophils, that were previously blocked, to migrate into the nearby wound region. Macrophages, which are essential phagocytic cells involved in wound healing, secrete enzymes such cytokines, tumor necrosis factor (TNF), interleukins, and collagenase during the process of phagocytosis. These enzymes aid in the removal of foreign substances and facilitate the growth of fibroblasts and angiogenesis. Macrophage-released VEGF and platelet-derived growth factor (PDGF) transition granulation tissue from the proliferative phase to the tissue regeneration phase. The resolution of the inflammatory phase plays a role in restricting the healing of chronic wounds, hence facilitating the ensuing proliferative phase. Secretome therapy, particularly focusing on its applications, offers numerous advantages in the regulation of vascularization and regeneration in wounded areas, such as muscle, nerve, and skin, as well as in nerve repair.[32]

Administration of secretome expedites the augmentation of phagocytosed cells, diminishes post-inflammatory fibrosis, and enhances the generation of new tissue. The decrease in pus volume in the wound following the second week of treatment suggests the involvement of neutrophil factor activity. This demonstrates that the secretome has an impact on the stimulation and reproduction of immune cells, and successfully sustains the equilibrium of anti-inflammatory and pro-inflammatory cytokine concentrations. Ormazabal's study shown a notable decrease in erythema, edema, and discomfort following a two-week secretome treatment. This validates the notable anti-inflammatory impact that has a good influence on the patient's quality of life. The secretome also has a significant impact on the manipulation of the extracellular matrix. This is supported by the observation that the conditioned media of the secretome from human umbilical cord blood inhibits the expression of matrix metalloproteinase (MMP)-1, which in turn reduces the breakdown of the collagen matrix and promotes the regeneration of fibroblasts. Within a controlled laboratory environment, the secretome also demonstrates its efficacy in accelerating the rate of wound healing by promoting the growth and multiplication of keratinocyte cells and skin fibroblasts.[33]

The secretome, when applied topically, has demonstrated its capacity to effectively permeate connective tissue, resulting in enhanced efficacy and collagen deposition, while also minimizing the likelihood of scar formation. Continuing research in this area indicates that the secretome holds promise as a novel treatment approach for a range of disorders,

including chronic wounds. The fundamental mechanism that drives the therapeutic effectiveness of the secretome in tissue regeneration is its capacity to generate diverse bioactive substances. These substances play a critical role in activating neighboring parenchymal cells and initiating essential processes involved in inflammation and tissue healing. Furthermore, it has been demonstrated that the secretome plays a role in controlling the immune system in the immediate vicinity, facilitating the growth of new blood vessels, inhibiting cell death, and triggering targeted cellular reactions such as cell survival, multiplication, and specialization of previously injured skin cells. This demonstrates the efficacy of the secretome in expediting the wound healing process, making it a promising treatment option for addressing persistent wounds in elderly patients.[28,34]

4. CONCLUSION

Recent advancements have greatly enhanced the understanding of the molecular mechanisms governing the natural process of wound healing, as well as the variables that impede this process. An integral part of this progress is the insight gained into the role of the stem cell secretome, which is enriched with growth factors like TGF-β, PDGF, KGF, FGF, Pro Collagen, VEGF, and exogenous factors. These components, as previously discussed, significantly accelerate wound healing by stimulating the proliferative and migratory abilities of skin cells. The findings of these and other investigations ultimately facilitate the development of more effective wound-healing therapies.Although proteases and inflammatory agents were once seen as impediments to the process of wound healing, it is now evident that their influence can be mitigated. This can be accomplished by integrating protease inhibitors into formulations that contain these vital growth factors or by utilizing truncated recombinant proteins that do not possess binding sites for proteases. With the progress in medical professionals' comprehension of growth factors, there exists the possibility of devising therapeutic approaches utilizing them. This method enables meticulous regulation of gene expression at the site of damage. While there are potential hazards associated with overexpression, a more comprehensive comprehension of the mechanisms governing growth factors, including those within the stem cell secretome, could aid in surmounting these obstacles.

CONSENT

Not Applicable

ETHICAL APPROVAL

Not Applicable

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