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Unraveling the Significance of Growth Factors (TGF-β, PDGF, KGF, FGF, Pro Collagen, VEGF) in the Dynamic of Wound Healing

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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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, proliferation, and remodeling. Any interruptions during this process can lead to wound healing that deviates from the

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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 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 contributions of stem cell secretomes 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 evolved extensively, developing 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 any tissue damage that disrupts its structure and function. The wound healing process primarily involves the restoration of damaged skin tissue. The wound-healing process commences immediately upon the injury of the epidermis and might persist for multiple years. This dynamic process encompasses highly coordinated mechanisms at cellular, fluid-based, and molecular levels [1,2]. The wound healing process can be categorized into three interconnected phases: inflammation, proliferation, and remodeling. Any interruption 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 and costly medical interventions for wound care impose a financial strain on the healthcare system [2,3].

Wound healing is an intricate biological phenomenon encompassing 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 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, a subsequent healing phase will ensue if the wound-healing process is interrupted by infection, wound reopening, inadequate oxygenation, or immune system abnormalities.

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 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 to enhance 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, 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 signaling 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, 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 healing efficiency and provide a favorable wound healing environment. Exogenous growth factors (eGFs) have been widely accepted and 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]. Nevertheless, the role of epidermal growth factor (eGF) has garnered significant interest. However, its efficacy remains an 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. MATERIALS AND METHODS

This literature review study aims to find database sources and primary information sources, including Scopus, EMBASE, PubMed, and Google Scholar. This database was selected based on its comprehensive compilation of peer-reviewed scholarly papers. Chosen publications must satisfy specific requirements, including those 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 wound healing. The research material must include original research articles, reviews, or meta-analyses.

The keywords and search phrases will be organized to capture 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, 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 wound healing. Information from multiple sources will be combined to create a logical and thorough account of the investigated topic.

3. RESULTS AND DISCUSSION

3.1 Wound Healing Mechanisms

The wound healing process has three interconnected stages: inflammation, proliferation, and remodeling, which occur sequentially [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 injury site. Simultaneously, the affected region

undergoes vasoconstriction, which narrows the blood vessels, for 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 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, a subsequent vasodilation phase follows. Vasodilation leads to augmented blood circulation towards the affected region, thereby inducing swelling (edema) in the wounded area. Blood circulation alterations and inflammation are the primary mechanisms of the inflammatory response to tissue injury [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 injury site and persist there for 2 to 5 days. Neutrophils have a crucial function in starting phagocytosis, which macrophages carry out. 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 also serve as chemoattractants for other cells and contribute to the intensification of inflammatory reactions by releasing several pro-inflammatory cytokines. Macrophages reach the injury site around 3 days following the initial tissue damage. Like neutrophils, they also secrete various 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: Granulation tissue formation and blood vessel tissue restoration characterize the proliferative phase. 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. 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 and 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 comprising type III collagen, glycosaminoglycans, and fibronectin. Granulation tissue comprises fibroblasts, granulocytes, macrophages, capillaries, and loosely organized collagen tissue [4,6].

Remodeling: The remodeling phase of wound healing, typically commencing 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 tissue creation and degradation, which happens continually. Any disruption to this process might lead to the development of chronic wounds. The granulation process is finished during this stage, and the wound enters the maturation phase. The extracellular matrix (ECM) undergoes specific alterations to enhance 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 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 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 occur. TGF-β1 induces fibroblasts to undergo differentiation into myofibroblasts. These myofibroblasts produce 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, 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 developing fully formed wounds 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 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 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 regenerate after 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 tissue above [4,9].

3.2 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 only sometimes full 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, preparing the wound site to promote skin regrowth in chronic wounds has been crucial in 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 over 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 wound care treatment as it prepares the wound site for re-epithelialization. Bacteria can obtain nourishment from dead tissue, which includes necrotic tissue. In addition, deceased tissue is 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 can obscure the true scope and seriousness of the damage, so concealing a possible underlying infection [3,9].

It is necessary to evaluate every wound thoroughly in terms of its status. This assessment entails a meticulous analysis of multiple factors, such as the wound's dimensions, its penetration level, and the existence of foreign bodies or infections. Thorough wound cleaning is crucial as it helps eliminate 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 influencing wound healing [11,12].

3.3 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 are pivotal 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 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. Researchers and clinicians have explored the potential of introducing external growth factors into non-healing wounds to overcome this challenge. The idea is to supplement the deficient local supply of these important molecules, hoping to improve cellular response and promote timely wound closure [11,12].

Transforming growth factor β: 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 in an inactive latent state. The N-terminal latent-associated peptide is attached to the C-terminal mature TGFβ noncovalently 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 the homeostasis of epidermal tissue and the various phases of wound healing. This engagement encompasses controlling diverse cell types, including keratinocytes, fibroblasts, endothelial cells, and monocytes. 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 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 vascular endothelial growth factor levels. Finally, TGFβ1 promotes the movement of keratinocytes, which is crucial for the process of wound healing [14,15].

Platelet-derived growth factor: PDGF displays various biological functions by interacting with target cell membrane receptors. These interactions initiate a cascade of biological responses pertinent to 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) is 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, stimulating 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 injury site. 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 extracellular matrix (ECM) components, 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 is a mediator 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 skin's healing process. 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) begin synthesizing KGF, which 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 skin's basal layer and 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 due to 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 can initiate the synthesis of crucial nucleotides required for DNA replication, RNA synthesis, and, subsequently, the proliferation of KCs throughout the wound healing process. 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 by breaking down proteins 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 originating 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, the 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 skin restoration process, hence enhancing wound healing. It stimulates cell division and proliferation (mitogenic activity) and decreases 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 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 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 before engaging in mitotic division. Capillary sprout growth can persist for 4 or 5 days via 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 wound healing and transformed into intricate structures. Collagen undergoes alterations in its kind, quantity, and organization during wound healing, ultimately determining the tensile strength of the healed skin. During the early stages of wound healing, type III collagen production occurs initially. However, this collagen is subsequently replaced by type I collagen, which is the skin's predominant collagen. Collagen is first arranged in a disorganized manner during the creation of granulation tissue. However, it later undergoes covalent cross-linking facilitated by the enzyme lysyl oxidase. This process refines collagen into a complex structure prepared to acquire tensile strength. The process of collagen restructuring persists for several months after the wound is closed, strengthening 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 and acting as a natural surface for cell attachment, proliferation, and differentiation. The biofilm formation triggers the activation of MMP-2 through microRNA, resulting in collagen degradation. This process predominantly leads to a decrease in the collagen I to III ratio. This hinders the biomechanical characteristics of healing skin, which could make it more prone to the reappearance of wounds. Recent analyses of collagen structure and function indicate that collagen fibrils assume a closed shape in healthy tissue that has been wounded. 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 skin elasticity and stabilizing growth hormones. Additionally, it helps regulate cell adhesion and communication between cells and the ECM. Through scar tissue remodeling over time, wounds that have fully developed eventually heal, forming 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 collagen fibrils' density, size, and orientation [10,28].

3.4 The Role of the Secretome in Wound Healing

The secretome, derived from mesenchymal stem cells (MSCs), has a crucial function in 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 can 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 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 utilizing secretome-based approaches have demonstrated encouraging outcomes by harnessing the regenerative capacity to 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 its versatile characteristics and potential application in numerous medical disciplines. Studies on the bone marrow-derived secretome (BM-Sec) have uncovered its function as a precursor differentiating into many cell types along the germline pathway, including fat, chondrocytes, and dense osteocytes. A recent study highlights the therapeutic effects of the secretome in regulating the milieu of tissues and the immune system's ability to respond, speeding up wound healing and decreasing the formation of fibrosis or scars. This therapeutic approach has been widely used in regenerative medicine 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 and 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 are pivotal in controlling the intricate wound-healing process. 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 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 VEGF

mediates the first process of secretome administration. 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 as cytokines, tumor necrosis factor (TNF), interleukins, and collagenase during phagocytosis. These enzymes aid in removing 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 regulating vascularization and regeneration in wounded areas, such as muscle, nerve, and skin, as well as in nerve repair [32].

The 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 impacts the stimulation and reproduction of immune cells and successfully sustains the equilibrium of anti-inflammatory and pro-inflammatory cytokine concentrations. Ormazabal's study showed a notable decrease in erythema, edema, and discomfort following a two-week secretome treatment. This validates the notable anti-inflammatory impact that positively influences the patient's quality of life. The secretome also significantly impacts 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 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].

When applied topically, the secretome has demonstrated its capacity to effectively permeate connective tissue, resulting in enhanced efficacy and collagen deposition while minimizing the likelihood of scar formation. Continuing research indicates that the secretome holds promise as a novel treatment approach for various 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 are critical in activating neighboring parenchymal cells and initiating essential inflammation and tissue healing processes. 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 and 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. As previously discussed, these components 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 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, the possibility of devising therapeutic approaches utilizing them exists. 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 AND ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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