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Mini-Review

Role of Macrophages During Skin Wound Healing in Terms of Angiogenesis

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Abstract

Angiogenesis is the establishment of new vessels by growing from previous ones in a developing or healing tissue for oxygen and nutrient supply and also removal of metabolic wastes. In order to develop a proper vasculature so many factors such as cells, growth factors, cytokines, enzymes have to function properly. One of the key factors in this process is macrophages which are involved in inflammation as well as angiogenesis. The disturbances of switching from that inflammatory to angiogenic character so called macrophage polarization may cause some abnormalities of healing and angiogenesis. In this review, we attempted to discuss the literature regarding macrophage polarization for angiogenesis during skin wound healing.

Keywords: Wound healing, angiogenesis, inflammation, macrophage polarization.

1. Introduction

Wound healing is a complicated process that involves 4 phases: homeostasis, inflammation, proliferation, and remodeling [1]. Each of these steps is composed of unique biological processes. Homeostasis is the phase including clotting of the blood around the wound by platelets. Then macrophages and neutrophils cause inflammation during the second phase which is the inflammation phase. During the proliferation phase macrophages, fibroblasts, keratinocytes, and lymphocytes are recruited to the wound area. In this phase reepithelialization, tissue granulation and angiogenesis occur. Lastly, fibroblasts come into play for collagen remodeling during the remodeling phase [2].

This review will be confined to explaining only major steps of the processes since wound healing and angiogenesis are reviewed in detail by other papers [3,4]. Briefly in angiogenesis (during the proliferation phase) the wounded tissue releases several growth factors and cytokines such as vascular endothelial growth factor (VEGF), angiopoietin-2 (Ang-2), and fibroblast growth factors (FGFs) which results with angiogenesis induction [5]. Additionally, endothelial cell (EC) proliferation, migration, and tube formation occur as a response to many growth factors (platelet-derived growth factor (PDGF), VEGF, transforming growth factor-α (TGF-α), TGF-β, and basic fibroblast growth factor (bFGF)) which are secreted by platelets [6]. PDGF, VEGF, Angiotensin-1 (Ang-1), TGF-α, bFGF, interleukin-8 (IL-8), and tumor necrosis factor-α (TNF-α) as proangiogenic factors are released by monocytes and macrophages [7].

Macrophages are involved in both inflammatory and proliferation phases of wound healing (Figure 1). TNF-α and interleukin-1-beta (IL-1β) are pro-inflammatory cytokines which are secreted by M1 macrophages in inflammatory phase [8]; while M2 macrophages release polyamines for induction of VEGF expression and collagen production [9]. If this polarization from M1 to M2 macrophage is distrupted then wound healing progresses improperly as in diabetic wounds [10]. This disruption slows down the speed of reepithelialization, granulation tissue formation, and vascularization [11].

2. Stem Cells Based Studies

Stem cells induce angiogenesis by two ways as differentiation to endothelial cells or release of extracellular vesicles [12,13]. In a study it is observed that when adipose derived stem cell (ADSC) spheroids are injected (inside a PNIPAM-based porous hydrogel) onto the wound region they improved wound healing by enhancing angiogenesis via M1-M2 macrophage transformation and thus production of anti-inflammatory cytokines [14]. In a study, the rats which had full-thickness excisional skin wounds were treated with xenografted human ADSCs in a fibrin glue matrix carrier. Researchers observed that wound healing is

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accelerated by enhancement of blood vessel numbers by increased VEGF expression and induced M2 macrophage profile. This result was attributed to increased IL-10 levels and decreased TNF-α levels. [15]. Apoptotic bodies (including microRNA-21–5p) which are extracted from adipose derived mesenchymal stem cells (ADMSCs) tested for wound healing in mice model and it is observed that these structures induced M2 macrophage polarization resulting with enhancement of granulation tissues and blood vessels. In this study it is confirmed by in vitro experiments that these effects are executed via targeting of KLF6 by miR-21–5p [16]. In a study where dermal stem cells (DSCs) and ADMSCs were applied to full-thickness mouse wounds via a collagen-based scaffold (Integra ® Matrix), it is observed that DSCs are exhibited better wound healing ability in contrast to ADMSCs regarding scar size, deposition of extracellular matrix, and number of cutaneous appendages. Both types of stem cells decreased the inflammation by inducing the M1 to M2 switch. But researchers revealed that DSCs produced better results in contrast to scaffold alone treatment while ADMSCs produced an intermediate effect but not much better than scaffold alone treatment [17].

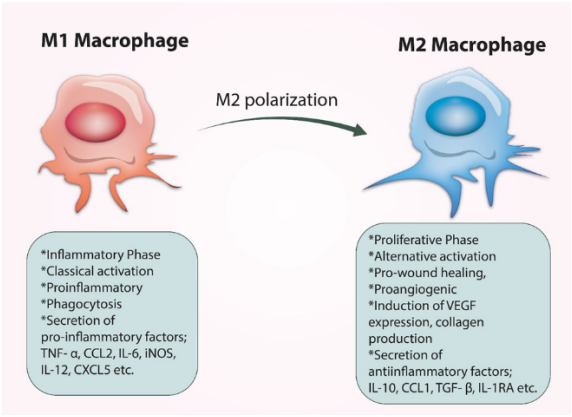

Figure 1. Differences of M1 and M2 macrophages

3. Biomaterials Based Studies

A group of scientists investigated the effect of thermosensitive injectable hydrogel (the thermosensitive decellularized adipose tissue/platelet-rich plasma inter-penetrating polymer network (t-DPI) hydrogel) on the healing of nude mouse full-thickness wound model. This hydrogel was based on decellularized adipose tissue (DAT) and temperature-controlled platelet-rich plasma (t-PRP). At the end of their study, they observed that this t-DPI hydrogel promoted angiogenesis and M2 macrophage polarization. They suggested that the effect of t-DPI hydrogel on M2 polarization originates from DAT [18]. A study resulted with increased diabetic wound healing angiogenesis when Chitosan@Puerarin hydrogel was injected on to the wounded area of miR-29ab1 knockout diabetic mice. This result is attributed to the inhibition of miR-29 that mediates prolongation of M1 macrophage polarization and elevated TNF-α and IL-1β levels in diabetic mice [19]. Decellularized Extra Cellular Matrix (dECM) integrated 3-D printed dermal analogs (PDAs) are tested as split thickness skin grafts and gave positive results in terms of reduced scar formation by inducing angiogenesis and M2 polarization. These results of PDA are associated with modulation of Wnt11, ATF3, and IL1β genes' expressions [20]. A hydrogel which is composed of gelatin methacrylate and microbial lipopeptide-surfactin (SF) together with the photoinitiator (GelMa-SF hydrogel) produced hopeful outcomes in diabetic wound healing. Gelma-SF hydrogel caused neovascularization based on higher levels of CD31 expression. Also, it is concluded that GelMa-SF hydrogels promoted diabetic wound healing by producing a lesser ratio of M1/M2 which is an indication of M2 polarization [21]. Hyaluronic acid (HA) hydrogel is combined with JK-1(a pH-controllable H2S donor) to form an HA-JK1 hybrid system. This hybrid system is tested for wound healing ability on mice full thickness removal wound healing models. This sytem improved wound healing by enhancing cell proliferation, angiogenesis, and macrophage polarization toward M2 phenotype in vivo. HA and HA-JK-1 treated groups showed nearly 2 fold and 4 fold higher amounts of vessels per mm2 subsequently in contrast to the control group [22].

4. Administration Based Studies

Phosphatidylserine-containing liposomes (PSLs) are tested for their ability to improve the healing of pressure ulcers modeled in young and aged mice. Subcutaneously injected PSL improved the number of vessels in both groups. Also, it is observed that PSL induced M1 to M2 polarization in vitro and in vivo. This material improved healing of pressure ulcers by enhancing myofibroblast-mediated ECM deposition, angiogenesis, and ultimately tissue remodeling. Also, the researchers experimented the effect of lipopolysaccharide (LPS) or LPS/IFNγ stimulation on naïve bone marrow derived macrophages (BMDMs) and they observed higher levels of TNFα, IL-6, and NO in middle aged mice group in contrast to young mice group. So, they concluded that aging modulates BMDMs inflammatory responses [23].

Some researchers examined the healing process of wounds on diabetic, obese, and hyperlipidemic mouse model (ob/ob) by using the administration of syndecan-4 and platelet derived growth factor-BB (PDGF-BB). They observed improved levels of re-epithelialization and angiogenesis in contrast to the PDGF-BB alone group. Also, this co-therapy induced enhancement of M2 macrophages but attenuation of M1 macrophages [24].

Selenium administration in diabetic mice improved wound healing through downregulating connexin gene expression, improving angiogenesis and antioxidant enzyme levels. Additionally, it is observed that selenium administration enhanced levels of VEGF on days 7 and 13 [25]. But in this study researchers didn't

examine the relationship between macrophage polarization and angiogenesis. I propose that it might be meaningful to examine this relationship of angiogenesis and macrophages via VEGF release since macrophages are able to produce VEGF as a proangiogenic factor [7].

When 5-aminolevulinic acid (ALA) is applied topically on the wounded skin of mice and then treated with photodynamic therapy (PDT) this combination (ALA-PDT) results in enhanced wound healing, thickened granulation tissue, reduction of inflammatory cell filtration (on 5th and 7th days), and the lesser ratio of M1/M2 macrophages. Also, ALA-PDT treated group showed increased anti-inflammatory cytokines Arg1 expression but decreased iNOS level as an indicator of M1 to M2 switch. In order to reveal the effect of ALA-PDT on macrophages western-blot analysis is performed and lesser amount of NF-κB phosphorylation is observed in vitro. This last result is the explanation of how ALA-PDT is inhibiting inflammatory responses. But in this study angiogenesis is not evaluated [26].

5. Conclusions

Macrophages are essential components of wound healing. A vast amount of research has been done but still, the equilibrium between inflammatory and angiogenic activity of macrophages couldn't be obtained by experiments for scarless and well vascularized wound healing. In the future with the aid of 4D biomanufacturing, it might be possible to establish smart biomaterials that could sense the tissue dynamics and change their behavior as a biosignal-producing machine for ideal wound healing.

Conflict of Interest

The authors declare no conflict of interest.

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