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Processes and Mechanisms Behind the Promising Stromal Vascular Fraction Treatment for Chronic Wounds.

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Abstract

Chronic wounds affect many people while a cure is not yet available. Current treatment aims for the prevention of infection rather or for the closure of the wound, but never both at the same time. At this moment, multiple treatments which aim for wound closure are being studied, such as ASC treatment, wound dressings, and most importantly, treatment with stromal vascular fraction (SVF).

SVF is generated from fat, can be retrieved by liposuction, and has, in previous studies (Bi et al., 2019; Bora & Majumdar, 2017), shown to positively affect wound healing. However, mechanisms responsible for the promising prospects of SVF are still unknown. For this reason, this study aims to understand which therapeutic components reside in SVF and secondly which mechanisms of wound healing are (re)initiated/boosted/reprogrammed by SVF components.

In this review, each cell component and the extracellular matrix (ECM) of SVF are discussed based on previous literature. As a result, cell-cell communications and activation of cells by cytokines or growth factors, which in turn activate cells within the SVF but also in the wound tissue, are described. The research shows the importance of the ECM as the provider of cytokines and growth factors but also as a communication hatch.

However, how long SVF is effective as a treatment, and the composition of the ECM of SVF needs to be studied. Overall, SVF treatment is very promising for the treatment of chronic wounds. The findings of this paper are summarized in figure 1.

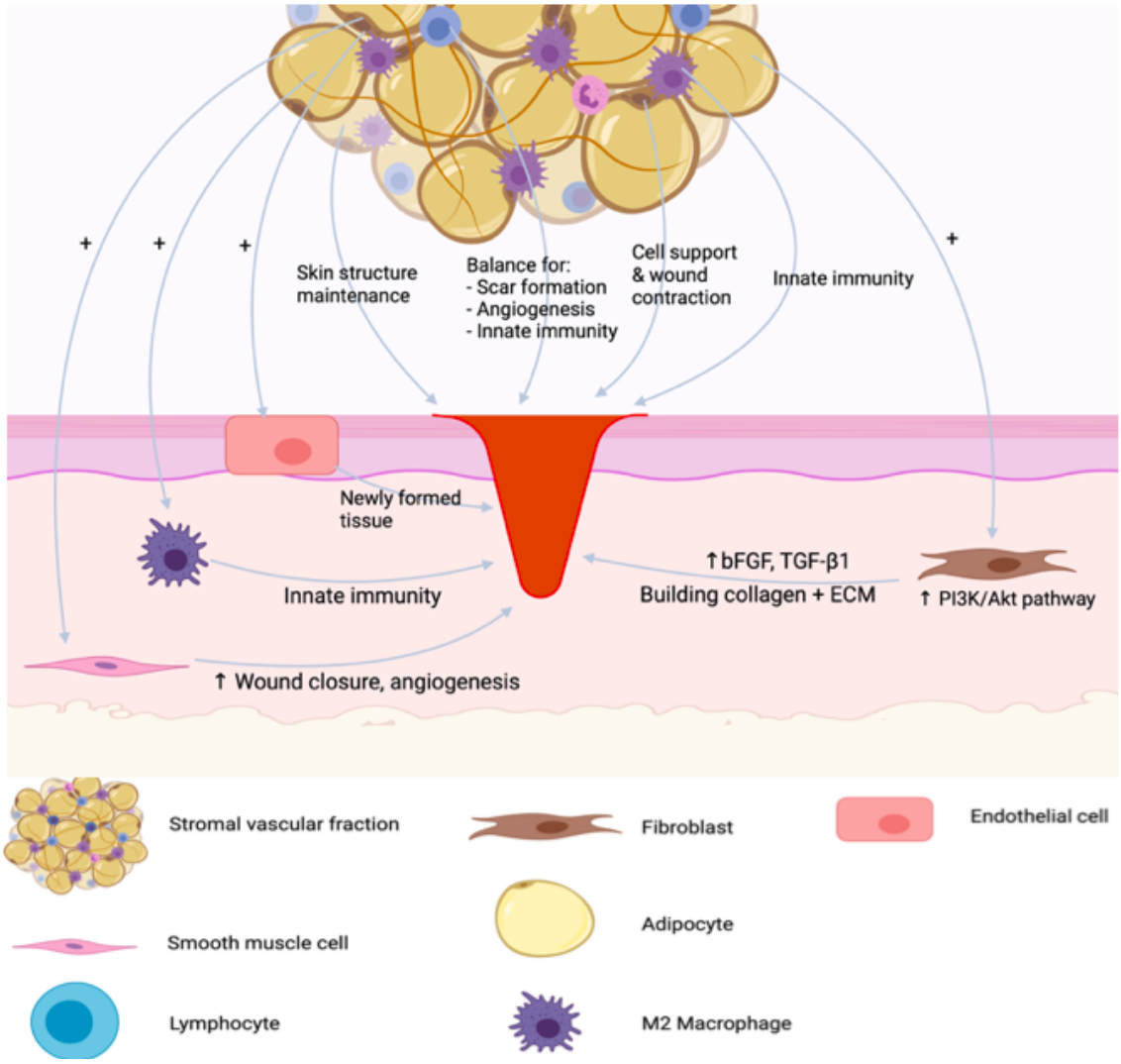

Figure 1. Graphical abstract created with BioRender.com.

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Introduction

One of the most important functions of the skin is wound healing since it prevents infections and tries to restore the function of the skin. The wound-healing process consists of four overlapping phases. The first phase is called hemostasis, this phase begins immediately after wounding. During hemostasis, the clot will form and the blood vessels connected to the wound will constrict so that blood loss is kept to a minimum. The clot is formed since the endothelium, collagen, and tissue factor activate platelet aggregation, which results in degranulation and release of chemokines and growth factors (GFs) (P. H. Wang et al., 2018). Then, the clot and tissue surrounding the wound also release pro-inflammatory cytokines and GFs, such as transforming growth factor-beta (TGF-β), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and epidermal growth factor (EGF) (Guo & DiPietro, 2010). These pro-inflammatory cytokines and GFs initiate the second phase, namely the inflammatory phase.

In the inflammatory phase, the wound site is infiltrated with neutrophils, macrophages, and lymphocytes. The neutrophils are responsible for providing a good environment for wound healing, by cleaning debris and bacteria at the injury site (P. H. Wang et al., 2018). Macrophages phagocytose to destroy and remove bacteria, foreign particles, debris, and damaged tissue (Velnar et al., 2009). The hemostasis and the inflammatory phase together approximately take 72 hours (P. H. Wang et al., 2018).

The third phase of the wound healing process is called the proliferative phase. In the proliferative phase, fibroblasts, keratinocytes, and endothelial cells are present in the wound. An extracellular matrix (ECM), which consists out of proteoglycans, hyaluronic acid, collagen, and elastin, and replaces the original clot. This phase also takes place under the influence of cytokines and GFs, such as the TGF-β family, interleukin (IL) family, and angiogenesis factors. The duration of the proliferative phase can be days to weeks (P. H. Wang et al., 2018).

Lastly, the remodeling phase will take place. During the remodeling phase degradation of redundant ECM and immature type 3 collagen are balanced with the formation of mature type 1 collagen (P. H. Wang et al., 2018). The remodeling phase could continue for months to years. To summarize, the four phases of the wound-healing process are schematically depicted in figure 2 (Garg et al., 2018).

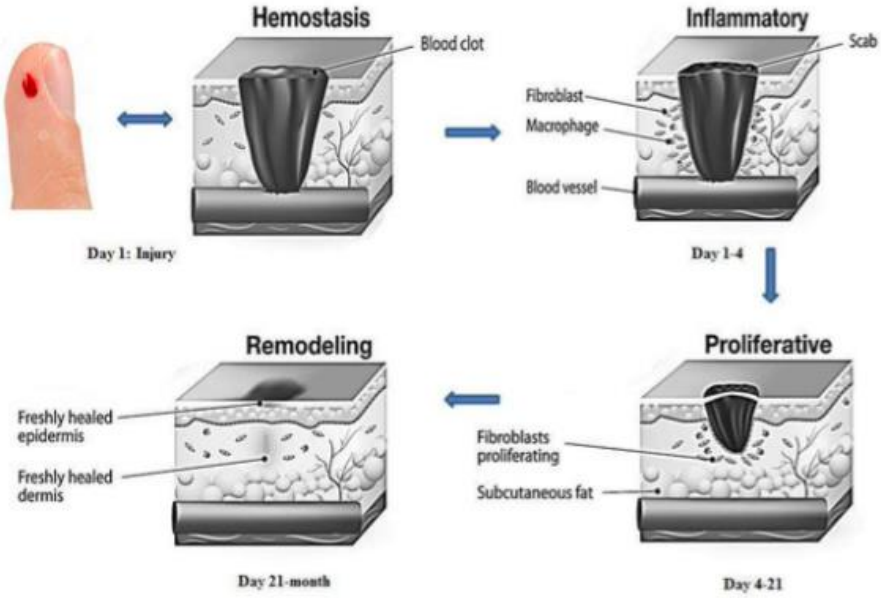

Figure 2. Schematic depiction of the four wound healing phases: Hemostasis, inflammatory phase, proliferative phase, and remodeling phase. From "A review on Nano-therapeutic drug delivery carriers for effective wound treatment strategies," by S. Garg, A. Garg, A. Shukla, S. K. Dev, and M. Kumar in *Asian Journal of Pharmacy and Pharmacology*, 4(2), 90–101. (https://doi.org/10.31024/ajpp.2018.4.2.1).

To go through all 4 phases successfully requires precise communication within and between cells. These communications work through different signaling pathways. The signaling pathways which are yet known to be responsible for dermal wound healing are among others AKT/mTOR (Jin et al., 2013), Wnt/β-catenin, Toll-like receptor signaling pathway (Portou et al., 2015), and Notch signaling pathways (Shi et al., 2015).

Even though wound healing is such an important process, proper wound healing is not self-evident for everyone. People who suffer from diseases such as diabetes mellitus and venous stasis disease often cope with chronic wounds, called ulcers (Guo & DiPietro, 2010). Diabetes and venous stasis disease both lead to impaired vascular flow, which leads to poor tissue oxygenation. Oxygen is important for important wound healing aspects, such as angiogenesis, re-epithelization, and wound contraction (Bishop, 2008). Thus, by decreasing oxygenation, diabetes and venous stasis disease impair the wound-healing process. Not only do diseases cause ulcers, but also too much pressure on the wound or ischemia could lead to a non-healing wound. Often, a non-healing wound is detained in the inflammatory phase of the wound healing process what causes tissue damage. The chronic inflammatory state is associated with excess neutrophil infiltration, which leads to the presence of reactive oxygen species (ROS) and destructive enzymes that cause the wound healing process to remain in the inflammatory phase (Zhao et al., 2016). This makes the implementation of the proliferative and remodeling phases a laborious process. These non-healing wounds affect about 6.5 million people in the United States, from which the majority are elderly (Richmond et al., 2013). In the Netherlands, approximately 350 thousand people are affected by chronic wounds (*Snellere genezing wond door unieke samenwerking,* 2017). Given that the number of diabetes mellitus cases is still increasing and the fact that people are getting older, it is expected that non-healing wounds will become a bigger problem in society. Thereby, are the costs of chronic wound treatment tremendous, since treatment costs around 1.5 billion euros in the Netherlands only (*Snellere genezing wond door unieke samenwerking,* 2017).

Currently, many treatments are available to help treat wound infections, called wound care. For wound care it is important that unhealthy tissue is removed, so that healthy tissue can proliferate and populate

the wound bed via epithelial cell migration (Han & Ceilley, 2017). This is often achieved by performing surgery or through antibiotics. In addition, products of collagen have already been used on chronic ulcers. The collagen products help facilitate an environment attracting cell types that are critical to wound healing, such as fibroblasts and keratinocytes. Despite promising aspects of treatment with collagen products, such as the stimulation of new tissue growth and re-epithelialization, do collagen products not promote the whole wound-healing process (Han & Ceilley, 2017). Therefore, more cells, next to collagen, are necessary for the treatment of chronic wounds.

There is also treatment available which, next to wound care, also tries to promote the wound healing process. These therapies are often referred to as wound dressings (Han & Ceilley, 2017). Different types of dressings exist, for example, collagens, hydrogels, foams, or alginates. Even though both in vitro tests and patient data show that wound dressings are promising, all dressings also come with multiple disadvantages. A significant drawback of many wound dressings is that the dressing might adhere to the wound bed which makes them difficult to remove. This could trigger pain and mechanical trauma or the dressing could disrupt the epidermis (Pilehvar-Soltanahmadi et al., 2018). There is even a wound dressing of hydrocolloids that is thick and yellow, causing it to be mistaken for an infection. Thus, conventional treatments have only moderate efficacy in improving chronic wound healing.

Recently, thorough research is performed for a new promising therapy, which uses stromal vascular fraction (SVF) as a promoter of wound healing. SVF is derived from the enzymatic digestion, and centrifugation of the product of liposuction, and thus consists of a variety of fat cells, mesenchymal stem cells (MSCs), vascular cells, and fibroblasts (Bi et al., 2019). Recent research by Bi et al. (2019), shows that SVF improves the function of endotheliocytes and fibroblasts, and regulates gene expression in the “cytokine-cytokine receptor interaction” pathway which together promotes wound healing. However, despite distinctive research with promising results, the mechanisms behind SVP are still unknown. Also, according to Bi et al. (2019), SFV seems a better alternative for wound healing treatment compared to adipose stem cell (ASC) treatment but the performance of all components in SVF, next to ASCs, is still unclear. The definition and isolation of SVF and SVF treatment in comparison to ASC treatment will be further described in the next paragraph.

Stromal Vascular Fraction

SVF is thus a heterogeneous cell mixture devoid of adipocytes. The cellular mixture primarily consists of adipose-derived stem cells, endothelial cells (ECs), pericytes, smooth muscle cells, monocytes, fibroblasts, and lymphocytes (Bora & Majumdar, 2017). These cells are all embedded in the extracellular matrix (ECM), only when SVF is retrieved through mechanical isolation. The ECM does not only contain these cell types but is also full of bounded GFs, which are needed for the first damage response for wound healing (Q. Li et al., 2017).

Isolation of SVF

In general, there are 2 ways for the isolation of SVF, namely through enzymatic isolation or through mechanical isolation. At this moment, the most widely used technique is enzymatic isolation, with collagenase. Enzymatic isolation is a form of intraoperative isolation. Collagenase can digest the collagen of the lipoaspirate using centrifugation, which separates the content into the mature adipocytes fraction and the cellular components of interest (Bora & Majumdar, 2017). In this way, the cells become disconnected from each other and the ECM is shattered, which causes the ECM to become absent. Next to the fact that a mixture with loose cells is undesirable, is this way of isolating SVF is also very time-consuming and requires a specialized culture lab (van Dongen et al., 2017). The mechanical isolation procedures are possible in two manners: One containing cell-cell communications (tissue SVF (tSVF)) and one which isolates single cells (cellular SVF (cSVF)) (van Dongen et al., 2017). The study by van Dongen et al. (2017), shows that the mechanical isolation results in even more volume of tSVF than cSVF from the enzymatic isolation procedure. Thereby, the ECM is still intact, cell-cell communications are preserved, and the procedure is less time-consuming. Overall, the intraoperative isolation of tSVF is a promising procedure but clinical safety still needs to be tested (van Dongen et al., 2017).

SVF vs ASC treatment

Next to SVF as a wound healing treatment, ASCs, which come from SVF, are becoming a promising treatment for chronic wounds according to different studies (Bi et al., 2019). However, ASC treatment has disadvantages compared to SVF treatment. Primarily, the isolation of ASC can take days to weeks since it requires an in vitro cell culture and expansion. Besides the fact that the isolation is time-consuming, all the steps for the isolation also increase the risk for infection by microorganisms and genetic mutations (Bi et al., 2019). This makes the clinical application of ASC more challenging than for SVF. Moreover, cells that are not embedded in the ECM, will migrate easily out of the tissue (Murasawa et al., 2008). In this way, it is expected that ASCs are less functional than SVF which is embedded in ECM and wherein cells have cell-cell communications. Bi et al. (2019) summarized the proposed molecular mechanisms for improved wound healing by SVF and ASC, which is depicted in figure 3.

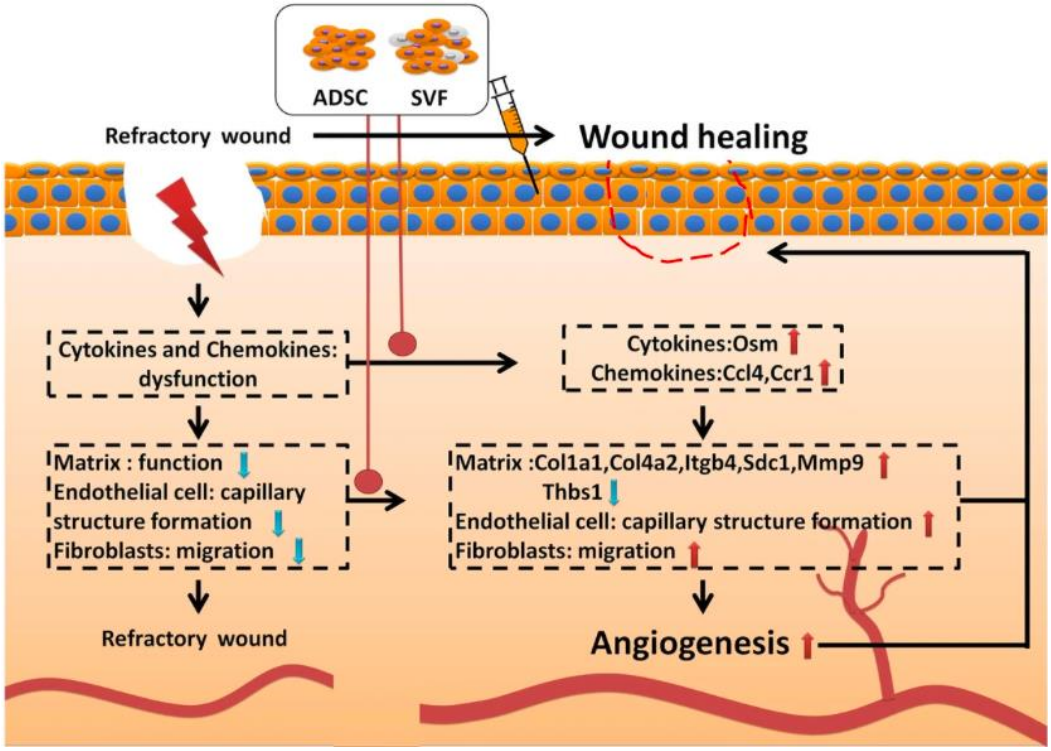

Figure 3. The proposed molecular mechanisms for improved wound healing by SVF and ADSC. From "Stromal vascular fraction promotes migration of fibroblasts and angiogenesis through regulation of extracellular matrix in the skin wound healing process," by H. Bi, H. Li, C. Zhang, Y. Mao, F. Nie, Y. Xing, W. Sha, X. Wang, D. M. Irwin, and H. Tan in *Stem Cell Research & Therapy*, 10(1). (https://doi.org/10.1186/s13287-019-1415-6).

How could SVF affect wound healing?

In a study by Bi et al. (2019) the mechanisms behind promoted wound healing as a result of cSVF treatment are studied. The findings showed that cSVF treatment results in the migration of fibroblasts, tubulogenesis of endothelial cells through regulation of cell adhesion, and improved collagen content of the skin (Bi et al., 2019). By using these mechanisms, cSVF promotes the wound healing process by stimulation of angiogenesis and matrix remodeling (Bi et al., 2019). Also, pathways were found which are differentially expressed as a result of cSVF treatment.

In this study, the different cells which are present in SVF will be examined to understand which therapeutic components reside in SVF and secondly which mechanisms of wound healing are (re)initiated/boosted/reprogrammed by these cells.

Each organ consists of parenchyma and stroma. The parenchyma is the active part of the tissue of an organ and has a specific function for each organ, in contrast to the stroma, which functions in every organ as supportive tissue. In this study, cells present in SVF are grouped to the kind of tissue they belong to and thus divided among parenchymal cells and stromal cells. SVF also contains a small blood vessel network. The cells in these blood vessels are also described.

Parenchymal Cells

Adipocytes

Adipocytes, in other words, fat cells, function as an active storage system, and thus belong to the active part of fat tissue. Adipocytes take up glucose and fatty acid from the blood, which is then converted and stored as triglycerides (TG). Furthermore, under fasting conditions, the adipocytes are responsible for breaking down stored TGs through lipolysis to produce energy in the form of free fatty acids (FFAs) and glycerol (Church et al., 2012).

A study by Schmidt & Horsley (2012) demonstrated that mice that lack mature white adipocytes, showed a lack of dermal fibroblasts. Wounds injected with an inhibitor for adipogenesis thereby showed reduced collagen production and a lack of fibronectin (FN) protein production. Moreover, 50% of the wounds of adipocyte-lacking mice re-opened as a result of a lack of a continuous epidermis over the wound bed (Schmidt & Horsley, 2013). Lastly, the study by Schmidt & Horsley (2012) showed that adipocytes influence fibroblast migration. These results suggest that adipocytes are necessary for fibroblast migration and are important in the reconstruction phase (Schmidt & Horsley, 2013). The main results of the study by Schmidt & Horsley (2013) are depicted in figure 4.

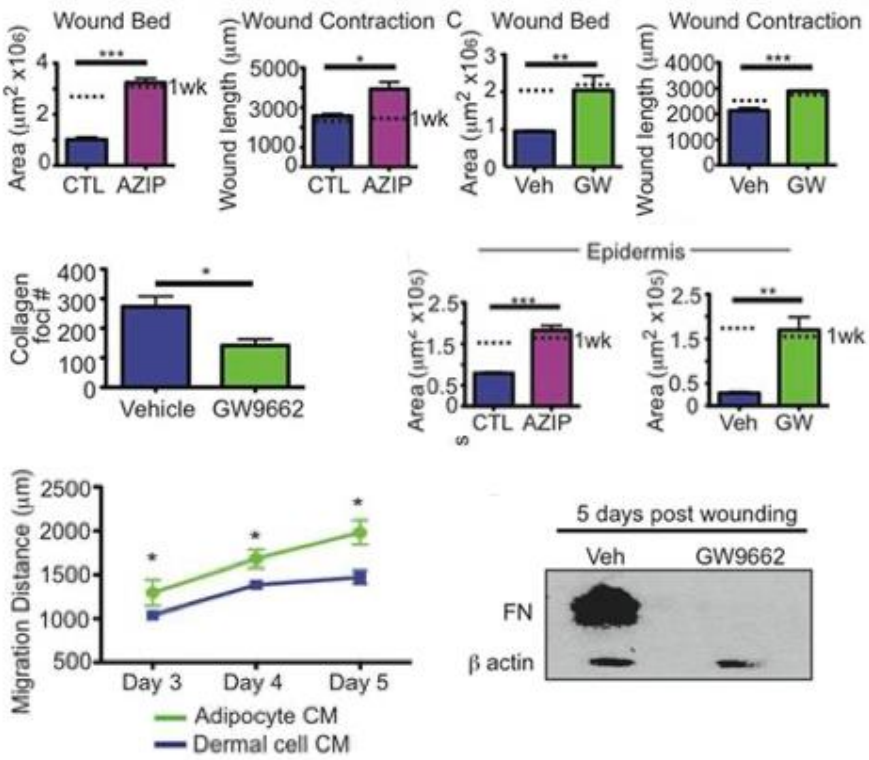

Figure 4. Main results for wound bed size, length of wound contraction, size of wound area, presence of collagen and FN, and migration distance of fibroblasts by mice which lack adipocytes (AZIP) and mice injected with GW9662, which is an inhibitor for adipocyte maturation. From "Intradermal adipocytes mediate fibroblast recruitment during skin wound healing," by B. A. Schmidt and V. Horsley in *Development*, 140(7), 1517–1527. (https://doi.org/10.1242/dev.087593).

One reason for adipocytes to be potentially essential for wound healing in SVF treatment is that adipocytes interact with macrophages via paracrine signaling (Kranendonk et al., 2014). One function of adipocytes in fat is initiating thermogenesis by releasing CXCL14. The release of CXCL14 stimulates M2 macrophage recruitment, which is the anti-inflammatory phenotype of macrophages (Y. Li et al., 2020). Thus, adipocytes present in SVF as a treatment for wound healing could lead to the recruitment or polarization of M2 macrophages. The M2 phenotype of macrophages is predominantly involved in the proliferative phase of the wound-healing process since M2 macrophages are

responsible for the migration and proliferation of the cells necessary for this phase, such as fibroblasts and keratinocytes.

SVF also contains the progenitors of adipocytes, namely ASCs. A study by Vishnubalaji et al. (2012) shows that ASCs have a significant role in maintaining the structure of skin tissue as a response to local injury. Thereby, ASCs can work as a rejuvenating mechanism for the skin, by sending younger cells to the outer layer of the epidermis (Mazini et al., 2020). However, these findings are still under discussion.

More specific research about the underlying mechanisms behind ASC, having such a significant role in wound healing, was performed by Zhang et al. (2018). This study demonstrates that ASCs release protein through exosomes but also direct via GFs, received by human dermal fibroblasts (HDF), which activate the PI3K/Akt signaling pathways in HDF. In turn, activation of the PI3K/Akt leads to more collagen type 1 and 3, and an increase in bFGF and TGF-β1, which both promote the wound-healing process and are summarized in figure 5. In general, exosomes contain proteins, including cytokines and growth factors, as well as signaling lipids and nucleic acids Zhang et al. (2018). Which cytokines, GFs, signaling lipids, and/or nucleic acids are present in ASC exosomes, and thus responsible for the activation of PI3K/Akt still remains unclear.

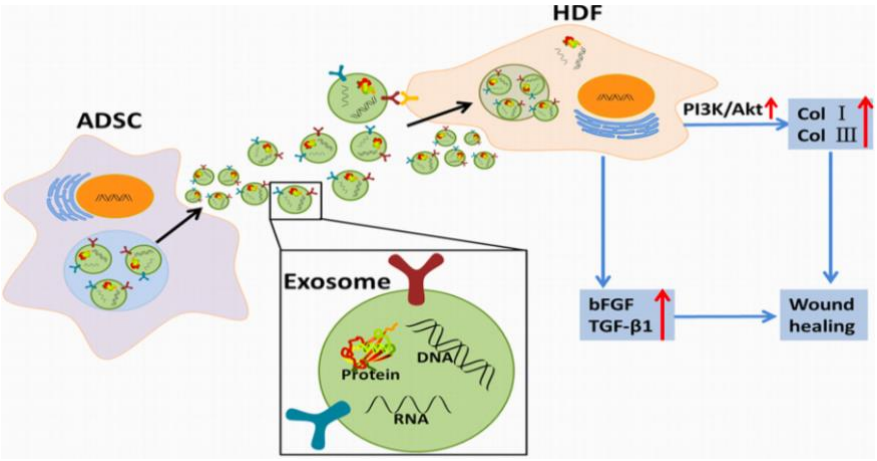

Figure 5. Schematic depiction of how ADSC promotes wound healing through activation of the PI3K/Akt pathway. From "Cell-free therapy based on adipose tissue stem cell-derived exosomes promotes wound healing via the PI3K/Akt signaling pathway" by W. Zhang, X. Bai, B. Zhao, Y. Li, Y. Zhang, Z. Li, X. Wang, L. Luo, F. Han, J. Zhang, S. Han, W. Cai, L. Su, L. Tao, J. Shi and D. Hu, in *Experimental Cell Research*, 370(2), 333–342. (https://doi.org/10.1016/j.yexcr.2018.06.035).

Also, activated PI3K/Akt signaling can lead to both an increase and decrease in mTOR (Jere et al., 2019). mTOR is known for its important role in dermal wound healing, functioning in the Akt/mTOR pathway (Jin et al., 2013). Thus, the activation of the PI3K/Akt signaling pathways in HDF can directly influence wound healing but can also influence wound healing indirectly by activating the Akt/mTOR pathway.

Stromal Cells

Macrophages

As stated in earlier paragraphs, M2 macrophages are important for the last stages of the wound-healing process. However, M1 macrophages and the precursor of macrophages, monocytes, predominantly play a role in earlier stages of the wound-healing process. This will be further explained in this

paragraph. Furthermore, in an earlier paragraph, it is hypothesized that M2 macrophages are recruited by the adipocytes in SVF treatment.

During hemostasis, M1 macrophages, which are pro-inflammatory macrophages, infiltrate and clean the wound of bacteria, foreign debris, and dead cells (Krzyszczyk et al., 2018). In non-chronic wounds, as the tissue begins to repair, the M1 macrophages start to transition into M2 phenotypic macrophages. The M2 macrophages promote the migration and proliferation of fibroblasts, keratinocytes and stimulate the endothelial cells to restore the dermis, epidermis, and vasculature (Krzyszczyk et al., 2018). This results in the closure of the wound and the production of a scar. However, in chronic wounds, there is no transition from M1 to M2 macrophages. Macrophages enter the tissue as their precursor, monocytes. Thus, in chronic wounds, monocytes can be transitioned to M1 macrophages but the transition from M1 to M2 macrophages does not take place. This makes the recruitment of M2 macrophages for wound healing by adipocytes in SVF very important.

Monocytes can be differentiated into macrophages, but also in dendritic cells, and are then recruited to the wound site to further amplify the inflammatory response (Krzyszczyk et al., 2018). The damaged tissue signals via damage-associated molecular patterns (DAMPs) or pathogen-associated molecular patterns (PAMPs) to recruit the monocyte-derived macrophages (Zhang & Mosser, 2008). Since the chronic wound will produce DAMPs and PAMPs when infected, migration of the monocyte-derived macrophages from the SVF to the wound site could be hypothesized.

M2 macrophages are normally polarized by interleukins IL-4, IL-13, and IL-10 (Alhamdi et al., 2019). These interleukins can be produced by several cells, among which CD4 lymphocytes (Junttila, 2018). Lymphocytes, which are also present in SVF, are able to produce IL-4 and IL-13 and thus should be able to indirectly activate M2 macrophages.

In short, M2 macrophages are generally not present in chronic wounds even though they are essential to the wound-healing process. However, M2 macrophages can be directly recruited by adipocytes in SVF and can be indirectly recruited by lymphocytes from SVF. Furthermore, monocytes from SVF can be differentiated into macrophages. Thus, SVF could be able to recruit M2 macrophages in 3 different ways and hereby promote wound healing. However, it can be debated whether the recruitment of macrophages by SVF is desirable. The macrophages could partly end up at the wound site but if the macrophages mainly migrate to SVF, they automatically will have less effect on the wound-healing process.

Lymphocytes

Correct functioning of the innate immune system is very important in wound healing, hence the importance of the M2 macrophages. Also, lymphocytes play a significant role in the immune system, predominantly for the adaptive immune system. When an antigen is presented through a histocompatibility complex (MHC) molecule, the innate system is triggered. As a result, lymphocytes respond to the foreign antigen when the adaptive immune system is activated (Wilson & Hunt, 2002). In figure 6, the activation of lymphocytes as a result of injury is depicted schematically.

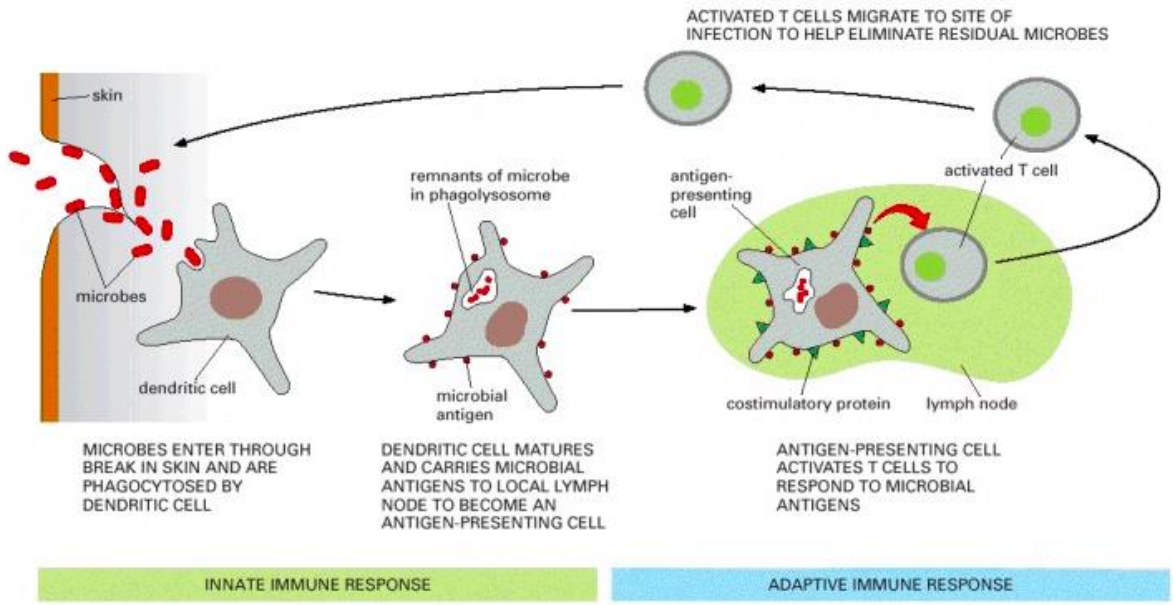

Figure 6. Schematic depiction of the activation of lymphocytes after injury. From "Molecular Biology of the Cell (4th ed.)," by J. Wilson and T. Hunt.

CD4+ T lymphocytes are also activated via an antigen-presenting cell (APC), suppress inflammation and collagen deposition to reduce scar formation in the early stage of the wound-healing process. The study by X. Wang et al. (2019) showed excessive scarring in lymphocyte deficient mice. This suggests that lymphocytes function as a brake on scar formation. Also, CD4+ lymphocytes activate other immune cells so that these immune cells release T cell cytokines and, in this way, modulate the immune response.

In addition, regulatory T lymphocytes (Treg cells), which express the FoxP3 gene, can suppress overactive immune reactions via interleukin 10 (IL-10), enable tissue tolerance, and restore homeostasis (X. Wang et al., 2019). Despite knowledge about the results of lymphocyte activation, is the regulatory contribution to scarring in the skin is not completely understood.

Thus, CD4+ T lymphocytes are key lymphocytes with regards to regulating a sufficient balance in scar formation, inflammation, and angiogenesis. These lymphocytes are thus very important players in SVF treatment and in the wound-healing process.

Mesenchymal Stromal Cells

Mesenchymal Stromal Cells (MSCs) have the ability to differentiate into adipocytes, chondrocytes, osteocytes, and fibrous tissue (Hu et al., 2018). ASCs are thus a type of MSCs. It has already been shown that ASCs have a positive effect on wound healing. Therefore, MSCs will have an indirect positive effect on wound healing too. In addition, MSCs play a role in the recruitment of fibroblasts and dermal reconstruction and are thus essential for wound healing (Schmidt & Horsley, 2013).

MSCs can be derived from different tissues, including bone marrow, embryonic and fetal sources, adipose tissue, and dermal tissue. Cutaneous wounds contain factors that activate the MSCs derived from adipose tissue, thus ASCs (Hu et al., 2018). Therefore, the factors present in the chronic wound could potentially work together with not only ASCs but also other MSCs present in SVF treatment.

It is known that chronic wounds are locked in the inflammatory phase of the wound-healing process. However, MSCs may provide a solution for this problem since wounds treated with MSCs already have shown to contain lower numbers of inflammatory cells and proinflammatory cytokines, such as

IL-1 and TNFα. Furthermore, as a result of MSC activity, less IFN-γ and more IL-4 are secreted by T cells and the number of Treg cells increases. As previously described, Treg cells are essential in preventing an overactive immune system and IL-4 is, among others, responsible for the activation of M2 macrophages, which are significant for the wound-healing process.

Thereby, less IFN-γ expression, as a reaction of MSC activity, has a positive effect on wound healing since the suppression of IFN-γ results in the production and functional activity of TGF-β1. IFN-γ and TGF-β1 can both antagonize one another, thus TGF-β1, in turn, inhibits IFN-γ production and its receptor expression (Ishida et al., 2004). The production of TGF-β1 is important for wound healing since TGF-β1 is involved in inflammation, fibroblast proliferation and differentiation into myofibroblasts, collagen synthesis, and deposition and remodeling of the new ECM (Pakyari et al., 2013). These processes are all essential for successful wound healing and thus could be reinitiated by SVF. Thereby, IFN-γ is an M1 macrophage driver, while M2 macrophages, as explained earlier, are more important for the treatment of chronic wounds. Less IFN-γ expression is thus desirable for the treatment of chronic wounds.

Extracellular Matrix

One of the final stages of wound healing is the remodeling of the ECM. ECM remodeling is a significant part of scar formation which can take months (Xue & Jackson, 2015). However, the function of the ECM of SVF lays differently since scar formation in SVF is undesired.

Based on previous paragraphs, the importance of cell-cell communications for wound healing became clear. The ECM plays a key role in these cell-cell communications by interaction with cells and growth factors what eventually leads to wound closure (Schultz & Wysocki, 2009). By using SVF as treatment, which contains an intact ECM, automatically growth factors and cytokines that are bound to the ECM are also present in the treatment.

According to studies, the following macromolecules that can be found in the ECM are essential for the wound-healing process: proteoglycans, glycosaminoglycans, collagen, and elastin (Maquart & Monboisse, 2014). In other words, all ECM components are essential for the wound-healing process. A schematic representation of how these macromolecules are build up in the ECM is depicted in figure 7.

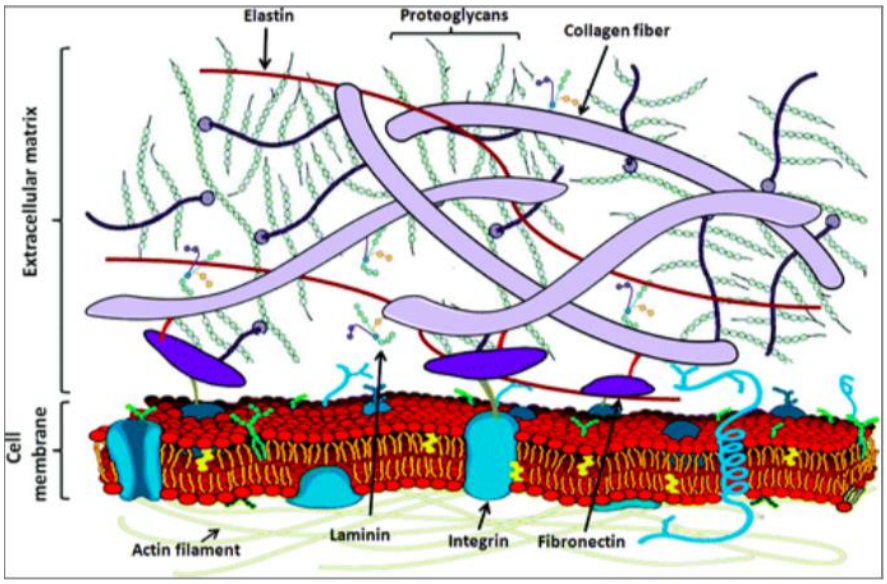

Figure 7. The ECM build up out of macromolecules. From "Extracellular Matrix Reorganization During Wound Healing and Its Impact on Abnormal Scarring," in *Advances in Wound Care*, 4(3), 119–136. (https://doi.org/10.1089/wound.2013.0485).

Proteoglycans play a big role in wound healing. The particularly important proteoglycans are the small leucine-rich proteoglycans (SLRPs) family, versican, perlecan, and syndecans (Maquart & Monboisse, 2014). LRPs can inhibit TGF-β, which stops the promotion of inflammation and fibroblast proliferation. This is important since excessive inflammation and fibroblast proliferation could lead to redundant scar formation. Furthermore, perlecan and versican are both important for angiogenesis, and syndecans are upregulated in keratinocytes and endothelial cells during wound healing to stimulate migration (Maquart & Monboisse, 2014). Perlecan can interact with vascular endothelial growth factor (VEGF), which stimulates endothelial cell proliferation and has been shown to stimulate the development of collateral arteries in human peripheral vascular disease (Segev et al., 2004). Thereby, enhances versican, especially the V2 isoform, angiogenesis by regulating endothelial cell activities and fibronectin expression (Yang & Yee, 2012).

Furthermore, the carbohydrate units of proteoglycans, namely glycosaminoglycans (GAG), are important for wound healing. Hyaluronic acid is one of the most significant GAG for wound healing and is responsible for the visco-elasticity and hydrophilicity of tissue (Maquart & Monboisse, 2014). Cell surface receptors in the wound will interact with hyaluronic acid coming from the ECM of SVF, which induces the wound-healing process, including modulation of inflammation, chemotaxis, cell migration, collagen secretion, and angiogenesis (Maquart & Monboisse, 2014).

Moreover, collagen is probably one of the most important molecules for wound closure. Collagen attracts fibroblasts and encourages the deposition of new collagen to the wound bed. Other studies which use collagen in a dressing already showed new tissue growth, by stimulation of autolytic debridement, angiogenesis, and epithelialization (Fleck & Simman, 2010). Also, studies showed that collagen can inactivate matrix metalloproteinases (MMPs), which are overly present in chronic wounds (Kyriakides et al., 2009).

Lastly, elastin, as the name says, is primarily responsible for the elasticity of the skin, but also plays a role in cell activities such as cell migration and proliferation, matrix synthesis, and protease production (Almine et al., 2012). Therefore, elastin is not only important for chronic wound healing, but also for the elasticity of wound healing of adult skin. Elastin being present in SVF is thus very desirable. Unfortunately, a study by Spencer et al. (2011) showed that the ECM of adipose tissue contains a low amount of elastin protein.

However, each tissue has a unique ECM composition and the components are described in the context of dermal ECM. Unfortunately, not much is known about the exact concentrations of these components in the ECM of fat tissue. Thus, the concentrations of the components present in SVF treatment require further studies.

Blood vessels

Out of a preexisting blood vessel in the adipose tissue, SVF can form a blood vessel network (Koh et al., 2011). In other words, SVF is able to induce and perform angiogenesis. These blood vessels contain several cells and factors, which could be a promising part of the SVF treatment. Cells present in these blood vessel networks of SVF, such as fibroblasts, smooth muscle cells, endothelial cells, and pericytes will be described.

Fibroblasts

Fibroblasts secrete many factors, including TGF-β, bFGF, insulin-like growth factor 1 (IGF-1), platelet-derived growth factor (PDGF), and epidermal growth factor (EGF) (Olczyk et al., 2014). First of all, TGF-β and bFGF are already described as essential growth factors for many processes regarding wound healing. Secondly, IGF-1 is pivotal for wound healing since IGF-1 increases protein production, cell proliferation, and migration (Caicedo & Devesa, 2019). Also, PDGF is important since PDGF can activate mesenchymal cells, including fibroblasts (Pierce et al., 1991). Lastly, EGF,

which is degraded in chronic wounds, plays a key role in stimulating epidermal and dermal regeneration (Bodnar, 2013).

Dermal fibroblasts, which reside in the wound itself, have a different function. In the wound, fibroblasts are essential for breaking down the fibrin clot as well as creating new ECM and collagen structures. By creating new ECM and collagen structures, fibroblasts ensure support for other cells (Bainbridge, 2013).

In short, fibroblasts are thus another type of MSC which are essential in SVF treatment since fibroblasts secrete multiple factors which promote wound-healing processes and activate other cells. At the same time, dermal fibroblast functions as ECM and collagen builders.

Smooth Muscle Cells

Vascular smooth muscle cells (MSCs) are not only important for the skin in the wound-healing process but are also needed for angiogenesis since SMCs reside in the walls of large blood vessels and blood itself. A study by Gorecka et al. (2020) has already shown that SMCs can accelerate diabetic wound healing, and are associated with increased cellular proliferation and improved dermal architecture and growth factor concentrations. Thereby shows the study by Gorecka et al. (2020) new blood vessel formation, an increase in M2 macrophages, and a decrease in M1 macrophages. The promising results of the study by Gorecka et al. (2020) are depicted in figure 8.

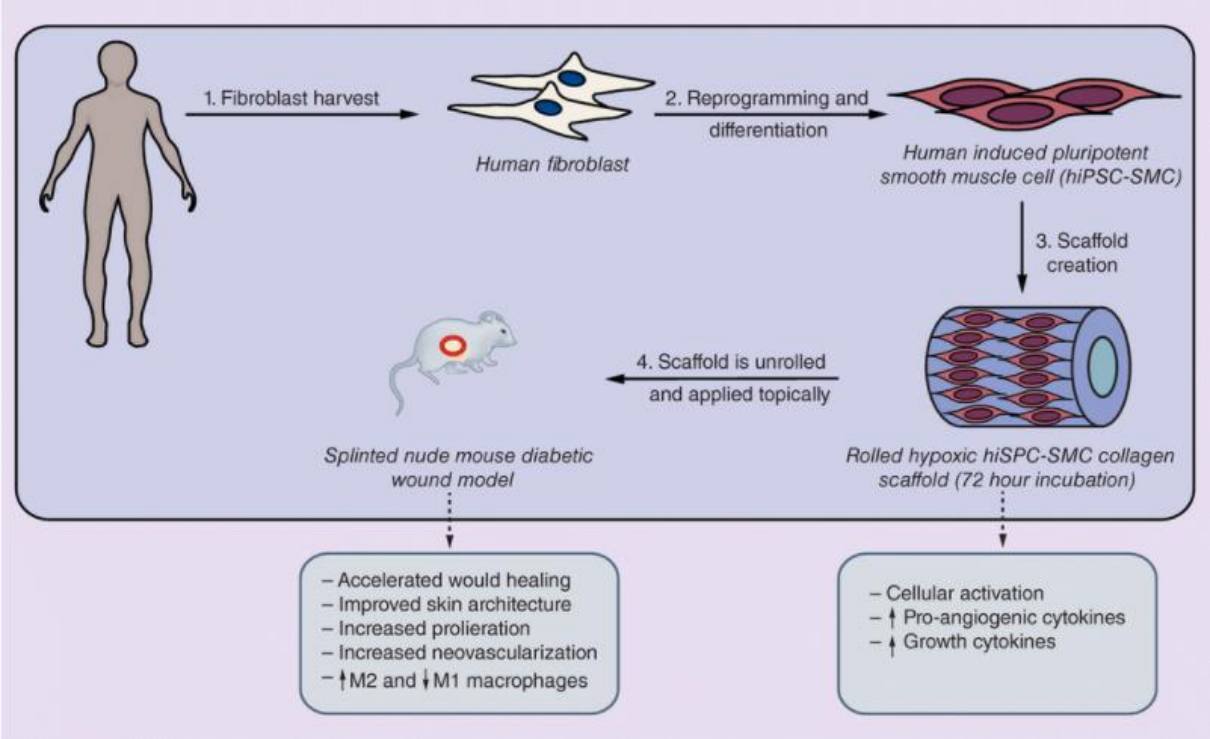

Figure 8. Main results after injecting a diabetic wound mouse model with SMC in a collagen scaffold. From "Induced pluripotent stem cell-derived smooth muscle cells increase angiogenesis and accelerate diabetic wound healing," by J. Gorecka, X. Gao, A. Fereydooni, B. C. Dash, J. Luo, S. R. Lee, R. Taniguchi, H.C. Hsia, Y. Qyang and A. Dardik in *Regenerative Medicine*, 15(2), 1277–1293. (https://doi.org/10.2217/rme-2019-0086).

SVF could activate SMC in the skin in different manners. First, through IGF-1 secretion by fibroblasts in SVF. IGF-1 is associated with inhibition of the synthetic SMC phenotype, and SMC proliferation and migration (Muto et al., 2007). Secondly, the SMC vasorelaxation is regulated by NO signaling. NO signaling, in turn, is linked to the Akt pathway while the Akt pathway is activated by ASCs (Muto et al., 2007). In this way, SVF is able to help regulate the functioning of smooth muscle cells.

Endothelial Cells

Endothelial cells are essential for the growth and survival of all newly formed tissue, which includes wound healing. Blood supply to tissues is dependent on endothelial cells (Velnar & Gradisnik, 2018) since signals from endothelial cells organize the growth and development of connective tissue cells that form surrounding layers of the blood vessel wall. Moreover, endothelial cells form a single cell layer that lines all blood vessels and regulates exchange between the bloodstream and surrounding tissues (Wilson & Hunt, 2002). Therefore, endothelial cells are pivotal for wound healing processes, including angiogenesis.

Activation of endothelial cells is necessary for their functioning. This activation can be done by several angiogenic factors which work in concert, including FGF, VEGF, PDGF, ANG, and TGF-α and -β (Velnar & Gradisnik, 2018). As described, bFGF, TGF-β, and PDGF are secreted by fibroblasts. Therefore, endothelial cells in SVF and in the wound can be activated by these factors secreted from fibroblasts in SVF.

Pericytes

Lastly, pericytes are pivotal for angiogenesis by stabilizing vessels, ensuring the blood barriers, and regulating flow through capillaries (Bergers & Song, 2005). Pericytes establish their functioning by communicating strongly with endothelial cells. TGF-β1 is activated by this communication, which leads to the inhibition of endothelial cell proliferation and migration, reduces the VEGF receptor 2 (flk-1) on endothelial cells, and induces differentiation of perivascular cells into pericytes. Thereby, TGF-β1 induces differentiation of MSCs and neuro crest cells into pericytes (Bergers & Song, 2005). Thus, pericytes from SVF could not only interact with endothelial cells in SVF but also with endothelial cells in the damaged skin. This can lead to tight communication between pericytes and endothelial cells, which helps to regulate wound healing.

Thereby, pericytes, another type of MSCs, have shown to have the ability to differentiate into ASCs (Xu et al., 2017). The importance of ASCs for wound healing has already been described in this review. Therefore, pericytes in SVF can indirectly promote the wound-healing process by differentiating into ASCs and can promote wound healing by playing an important role during angiogenesis.

Discussion

This review explains why SVF seems a promising treatment to promote wound healing and how SVF could promote wound healing. Particularly, in combination with the isolation method by Van Dongen et al. (2017), to keep the ECM intact. This review shows the importance of the ECM as part of the treatment. Therefore, the use of tSVF instead of cSVF as a treatment of chronic wounds is highly recommended. However, there are still factors that need to be considered.

First of all, as already partly described, the extent to which growth factors and cytokines are present in the ECM of SVF is still undiscovered. In general, the ECM of each tissue is composed of water, proteins, and polysaccharides. Each tissue has a unique composition and topology of these proteins and polysaccharides. Over- or under-expression of growth factors or cytokines might lead to unwanted cell behavior. The fact that both in vivo and in vitro experiments showed promoted wound healing without any unwanted cell behaviors, suggests that the composition of ECM and the concentration of cells present in SVF might be sufficient for treatment. However, the concentration of the described cells in SVF still might differ from the concentration of the same cells in the dermis. Thereby, studies for SVF as a treatment for chronic wounds have not taken place yet.

Second, there is no research performed on the longevity of SVF treatment. Cell migration is inevitable, causing the treatment to be less functional. As described earlier, it is expected that SVF will at least be functional for a longer period compared to ASC treatment. However, whether this period is sufficient for the healing of chronic wounds still needs to be studied.

Thus, more studies need to be performed to map the longevity of SVF and to look into the ECM composition of fat tissue compared to ECM dermal tissue. Furthermore, clinical trials should be performed to support efficacy, map possible side effects, and long-term outcomes.

Conclusion

In this research, based on thorough literature research, many processes and mechanisms which could be initiated by SVF are described. What stands out is the tight communications between cells, growth factors, and cytokines, which are required for wound healing. Furthermore, the contribution of SVF to these tight communications became clear, mostly because of the broad composition of SVF. Moreover, results show the importance of an intact ECM in order to provide growth factors and cytokines and to make cell-cell communications possible.

In short, SVF, in particular tSVF treatment, is very promising for the treatment of chronic wounds. Each component of SVF has its own contribution in promoting wound healing, which, besides the fact that SVF can be very easily isolated, also makes SVF a better alternative compared to ASC treatment.

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