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Applications of human amniotic fluid stem cells in wound healing

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Abstract
Complete wound regeneration preserves skin structure and physiological functions, including sensation and perception of stimuli, whereas incomplete wound regeneration results in fibrosis and scarring. Amniotic fluid stem cells (AFSCs) would be a kind of cell population with self-renewing and non-immunogenic ability that have a considerable role in wound generation. They are easy to harvest, culture, and store; moreover, they are non-tumorigenic and not subject to ethical restrictions. They can differentiate into different kinds of cells that replenish the skin, subcutaneous tissues, and accessory organs. Additionally, AFSCs independently produce paracrine effectors and secrete them in exosomes, thereby modulating local immune cell activity. They demonstrate anti-inflammatory and immunomodulatory properties, regulate the physicochemical microenvironment of the wound, and promote full wound regeneration. Thus, AFSCs are potential resources in stem cell therapy, especially in scar-free wound healing. This review describes the biological characteristics and clinical applications of AFSCs in treating wounds and provide new ideas for the treatment of wound healing.
Keywords: Amniotic fluid; Stem cells; Mesenchymal stromal cells; Wound healing; Cicatrix; Hypertrophic; Tissue engineering

Introduction

Wound healing is a complex and dynamic three-phase process comprising inflammation regulation, cell proliferation, and tissue remodeling. These processes are tightly regulated by the actions of various cytokines, chemokines, and growth factors.[1] Disruption of these processes leads to delayed wound healing and scar formation. Stem cells undergo self-replication, differentiate into multiple cell types, and affect paracrine signaling in the wound environment, and all these attributes are crucial for scarless tissue renewal and regeneration after injury. Stem cell-based therapies have emerged as a promising new approach in regenerative medicine.[1]

The embryonic stem cells (ESCs) are derived from early embryonic tissues and have been described as pluripotent stem cells. ESCs have almost unlimited proliferation potential and are considered to be an ideal source of stem cells. However, the application of ESCs for clinical research is limited due to ethical concerns. The induced pluripotent stem cells (iPSCs) are considered as pluripotent stem cells with strong proliferation ability similar to ESCs. However, the process of induction and transformation of iPSC may lead to mitochondrial DNA damage and

DNA mutation; at the same time, iPSCs have higher technical and cost requirements than ESCs and AFSCs.[2,3] Fauza[4] found that the AFSCs shedding in the amniotic cavity primarily originate from the developing fetal skin, amniotic membrane, respiratory apparatus, or urinary and gastrointestinal tracts. There are also a small number of stem cells that are derived from early embryonic or extraembryonic tissues,[4] both of which have strong proliferation ability and differentiative potential than hematopoietic stem cells and other adult stem cells,[5] that may play an important role in promoting wound healing. Additionally, AFSCs do not have the same ethical and legal restrictions as ESCs.[6] As medical wastes, the stable cell lines of AFSCs can be obtained via amniocentesis during the pregnancy intermediate stage or cesarean section.[3] Compared with ESCs and iPSCs, the ability of AFSCs to multi-differentiate is limited, but they do not have the risk of teratoma formation.[7] In addition to these desirable properties, AFSCs participate in immunomodulation, regulate inflammation, and are universally histocompatible.[8] A comparison of cells from the amniotic fluid (AF) and amnion has shown that the expression of pluripotency markers is higher in AFSCs than in homologous cells isolated from the amnion.[9] Moreover, AFSCs are the least senescent source of mesenchymal stromal cells and have the longest telomeres, supporting

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their robust proliferative potential.[10] Therefore, AFSCs are an attractive tool for tissue engineering and regeneration. This review explores the role of AFSCs in wound healing by describing the properties of AFSCs and their utility in stem cell therapies.

Biological properties of AFSCs

The AF surrounds the growing fetus within the amniotic cavity, which is a clear, watery liquid. It contains heterogeneous and multipotential cell types, which are populations with varying morphologies, *in vitro* characteristics, *in vivo* potential, cell surface markers, and biochemical properties. The constituents and volume of AF change depending on the stage of gestation and whether the fetus has any diseases during pregnancy.[6] Generally, it is recommended that relevant examination should be carried out before collecting AF from healthy pregnant women, to avoid AF from patients with metabolic diseases such as diabetes mellitus. Researchers must also ensure that the AF is free from viral (hepatitis B, human immunodeficiency virus, and so forth) and pathogenic bacterial infection.[11] Studies have shown that the age of pregnant women also affects the proliferation of AFSCs. In women aged 20 to 29 years, 30 to 39 years, and 40 to 49 years, the proliferation doubling times were 30.9 h, 38.3 h, and 43.9 h, respectively.[12] Rossi *et al*[13] reported that AFSC yields from the first trimester were higher than that of the second and third trimesters; however, the changes in marker expression were only observed in different cell subpopulations instead of cells from different trimesters. Based on the expression of the cell lineage markers, numerous mesoderm and endoderm cells are present during early gestation. In contrast, an equal number of ectodermal cells have been detected in AF from early and late gestational periods.[14]

Even approximately 2 to 5 mL of AF obtained via amniocentesis in the second trimester of pregnancy during prenatal gene screening can still be used to extract and subculture AFSCs.[15] The number of AFSCs increased from 1×10^5 to $(6.4 \pm 2.3) \times 10^9$ cells after 1 month and with being sub-cultured 10 times.[16] In contrast, more AF volume can be obtained during cesarean section at the end of gestation; the extraction operation is simple, reduces medical waste, and poses no risk to the mother or child. Additionally, the use of AFSCs does not violate any ethical guidelines because AFSCs can be harvested without harm to the fetus.[17] Compared with AFSCs harvested in the second trimester, AFSCs obtained in the perinatal period are similarly rich in neurotrophic, immunomodulatory, anti-fibrotic, and endothelial stimulating factors.[15] These advantages greatly improve the availability of AFSCs.

Early research findings showed that AF generally contains three types of cells: AF-type, which are mainly used for prenatal diagnosis; epithelioid-type, which secrete various neurotransmitters and cytokines; and fibroblastic-type, which have strong proliferation ability.[18] However, the percentages of specific cell types reported varies in different studies. Most of these cells are non-adherent cells and could be typically cleared away during the process of cell culture, while stem cells can be selected using one-stage or two-stage culture because they can attach to the cell bottle. AF-derived

MSCs (AF-MSCs) and AFSCs are the two kinds of stem cell populations in the AF. Of the two, AF-MSCs are easier to isolate and are abundant, whereas AFSCs account for <0.9 to 1.5% of AF cells.[19] During cell culture, these stem cells display a whirlpool-like monolayer adherent growth pattern seen in fibroblast cells. The AFSCs usually have a higher number of potential stem cells, leading them to express more pluripotent stem cell-related markers, and greater differentiation potential than AF-MSCs. Different cell subsets and populations of AFCs can be isolated using cell sorting or immunomagnetic separation with different types of surface markers, such as c-kit or CD-117 [Figure 1].[20,21] However, a phenomenon occurs in which different subgroups of AFSCs respond to one kind of stem cell surface marker. Recent studies have shown that Celector® technology should be used in precise analysis such as genome sequencing. It has also been used to further screen the biological characteristics of different subsets of AFSCs.[22] In a previous study, gene set enrichment analysis was used to identify the different subgroups of AFSCs that have significant differential expression in pathways related to stemness, DNA repair, E2 transcription factor targets, G2-M checkpoints (G2/M phase checkpoint which transition from gap-phase [G2 phase] to mitotic phase [M phase]), hypoxia, epithelial-mesenchymal transition, mammalian target of rapamycin 1 (mTORC1) signaling, unfolded protein response, and p53 signaling.[22]

Most AFSCs are stromal cells that contain some stem cells derived from the early embryo and extraembryonic tissues. They proliferate rapidly, can be easily expanded into stable lines *in vitro,* and have also been considered as ideal candidates for iPSCs.[3,6,23] Studies in *vitro* have shown that AFSCs can differentiate into hematopoietic, neurogenic, osteogenic, chondrogenic, adipogenic, renal, and hepatic germ lineages.[24] They express pluripotency-related markers: *Oct4, Nanog, Sox2, SSEA4*, and do not express hematopoietic stem cell-related surface markers, such as *CD34* and *CD45,* Nanog is vital in delaying cellular senescence, increasing endogenous expression of the embryonic genes *Oct4* and *Sox2, and* maintaining stemness and self-renewal.[25] Different subgroups of AFSCs may have complex and varied surface markers.[26,27] The phenotypic characteristics of AFSCs vary with the stage of pregnancy,[3] and epigenetic modifications guide AFSC differentiation into multiple lineages, playing a vital role in stem cell fate determination. Differentiation of AFSCs is regulated by DNA methylation, histone modification, and the expression of small non-coding RNAs,[28] most notably the *miR-351-3p, a* gene that targets *Abca4* (ATP-binding cassette transporter A4, Abca4) and regulates the proliferation and migration of AFSCs by targeting the 3′-UTR (untranslated region, UTR) of *Chrdl1* (chordin-like 1, Chrdl1) and downregulating its expression.[29,30]

Reports have shown that AFSCs grow well in a serum-free medium, and the proliferation efficiency was higher in the AmnioMAX medium than in Dulbecco's modified Eagle medium.[12,31] The cells have a doubling time of about 36 h, can proliferate 250 times *in vitro* without chromosome loss,[3] and maintain stable telomere lengths over several generations. When cultured in a medium used for ESCs, AFSCs proliferate rapidly, differentiate extensively, and
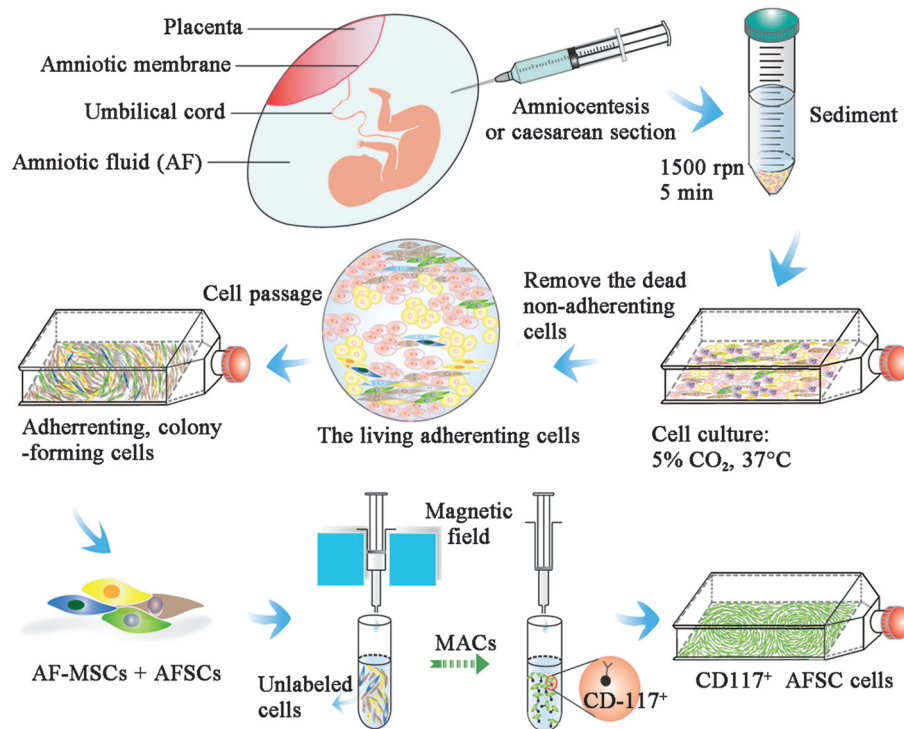
Figure 1: The heterogeneity and purification of AFSCs. AF contains various cell types. After several passages, only attaching and colony-forming cells can continue to survive. These cells are heterogeneous and been called AFSCs, can be further screened and purified by magnetic cell sorting (MACs). AF: Amniotic fluid; AF-MSCs: AF-derived MSCs; AFSCs: Amniotic fluid stem cells; MACs: Magnetic cell sorting.

revert to their undifferentiated phenotype in response to the transforming growth factor-β (TGF-β).[32] Furthermore, freezing does not result in aberrant chromosomal content for AFSCs, and the cells remain viable when frozen in 5% dimethylsulfoxide and 10% fetal bovine serum.[33] Human AFSC migration and proliferation is enhanced by sphingosine-1-phosphate (S1P).[34] Additionally, hypoxia stimulates AFSC differentiation and inhibits AFSC senescence.[34,35]

The genomic stability and epigenetic fidelity of AFSCs are high. De Coppi *et al*[10] injected AFSCs into the hind leg muscles of nude mice and found no evidence of teratoma formation after 3 months. This might be associated with the high expression of tumor suppressor p53, which controls cell proliferation, differentiation, and apoptosis, by AFSCs.[36] Moreover, this tumor suppressor is primarily expressed in the AFSC nuclei, where it maintains genomic stability and is an essential regulator of stem cell fate.[36] Furthermore, mammalian target of rapamycin 1 is also associated with the non-tumorigenicity of AFSCs.[37]

Application of AFSCs in treating wounds

AFSCs and scar-free wound healing

The science of how a wound heals is fascinating, to find the treatment scheme to promote rapid and scar-free wound healing. Researchers have carried out a lot of research.[38]

Rowlatt[39] did not observe scar formation in the skin of a 20-week-old human fetus after trauma. Further research about the differences among scar formation or not have been carried out. Research finding that scar-less fetal wound healing is associated with shorter periods of inflammation, lower macrophage count and activation, higher fibroblast migration, higher expression of hyaluronic acid receptors, and different growth factor profiles when compared with adult wound healing with scarring.[1] Fetal dermal fibroblasts synthesize more type III collagen than adult stem cells, and their collagen fibers form a lattice that facilitates dermal regeneration (including epidermal appendages) and minimizes fibrosis.[40]

Recent studies have found that AFSCs have a unique ability to accelerate skin wound healing and reduce fibrotic scar formation. Human AFSCs in the conditioned medium of keratinocytes can promote wound closure by differentiating into keratinocytes[20] and secreting paracrine signals via the TGF-β/SMAD2 (Mothers Against Decapentaplegic Homolog 2, Smad2) pathway. This aids the regulation of the rich extracellular matrix (ECM), which expresses high levels of anti-fibrosis mediators. In addition, this remarkably reduces type I collagen bundles in the wound and increases the level of type III collagen, but does not affect the size of the granulation tissue area.[20,41] Fukutake *et al*[20] found that 1×10^6 human AFSCs, suspended in phosphate-buffered saline (PBS) or cell-free PBS and injected into the full thickness of the skin defect wound with a size of $1 \, cm^2$, were observed in the dermis until day 7. They also found that α-smooth muscle actin-positive myofibroblast expression was suppressed in the dermis at the transcriptional level.[20] The process was characterized by lower numbers of myofibroblasts and higher expression of type III collagen. This implies that human AFSCs improve the quality of ECM

remodeling and promote wound closure independent of wound contraction by regulating fibroblast differentiation into myofibroblasts.[20] Therefore, AFSCs can accelerate skin wound healing by promoting re-epithelization of the epidermis without affecting the granulation tissue area in the dermis.

Immunomodulatory effect of AFSC

Human AFSCs have low immunogenic, anti-inflammatory, and immunomodulatory properties. The apoptosis of lymphocytes induced by AFSC-derived exosomes decreases the proliferation of lymphocytes.[42] Early studies suggested that T-cell activation depends on both cell-to-cell contact and humoral or paracrine factors, such as indoleamine-2,3-dioxygenase, galectin-1, prostaglandin E2, and B7-H (B7 family member homologue).[43] It has been shown that AFSCs inhibit immune hyperactivity by secreting exosomes and cytokines interleukin (IL)-4, IL-10, and IL-17,[43] which inhibit natural killer cells and CD8+ T-cell activity and promote T helper 2 cell polarization, Th1 cell inhibition, and Th17 cell induction.[43] This also prevents dendritic cell differentiation and maturation and promotes regulatory T-cell (Treg) activity in the local tissue niche.[43] AFSCs also promote the conversion of pro-inflammatory M1 (CD80) macrophages to the anti-inflammatory M2 (CD163 and CD206) phenotype and reduce the secreted levels of interferon-γ (IFN-γ), which influences inflammation and fibrosis during tissue remodeling.[44,45] In addition to the bioactive substances carried by exosomes, membrane surface proteins also play an important role in immune regulation. Among them, 5′-nucleotidase (CD73) converts adenosine-triphosphate (ATP) with immune activation into adenosine monophosphate and adenosine. Adenosine binds to the A2A receptor of adenosine on effector T cells, resulting in increased T-cell apoptosis.[46]

Sun et al[16] reported that human AFSCs did not directly secrete repair-related factors, such as fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), stromal cell derivedfactor-1 (SDF-1/CXCL12), TGF-β1, and Keratinocyte growth factor (KGF). However, B7H4 (B7 family member homologue 4), a negative costimulatory molecule, regulates low immunogenicity by reducing the expression levels of IL-1β, IL-6, tumor necrosis factor-α (TNF-α), cyclooxygenase 2, and membrane attack complex3, providing a moderately low-inflammatory microenvironment and initiating and promoting the early repair of skin injury. A recent study has shown that members of the B7:CD28 costimulatory family and the programmed death 1 receptor, as well as PD-ligand (PD-L) signal transduction, inhibited PI3K and ATP-dependent tyrosine kinases (AKT) activation. This decreased T-cell proliferation, cytokine production (IL-2, IL-6, TNF-α, IFN-γ), and cell survival; inhibited T-cell responses, and promoted peripheral immunotolerance.[47]

Culturing AFSCs with IFN-γ increases the expression of enzymes Indoleamine 2,3-dioxygenase 1 (IDO1) and PD-L1. These conditions immunomodulate the local environment, causing a considerable decrease in T-cell proliferation and

an increase in the numbers of CD4+CD25+FOXP3+ Treg.[47,48] IDO1 expression confers immunotolerance to recipient cells via mechanisms involving the kynurenine-dependent fostering of Treg-cell differentiation.[49] These mechanisms calibrate Treg functions, inhibiting inflammation and fibroblast migration.[50] The S1P and TGF-β1 in the AF immunomodulate AFSCs; S1P promotes the migration, differentiation, and immunomodulation of AFSCs, whereas the combination of S1P and TGF-β1 stimulates human AFSCs to express IDO1 [Figure 2].

AFSCs in promoting wound repair

#### Epidermal differentiation and paracrine effect of AFSCs

AFSCs promote wound healing by differentiating into all the cell types needed to regenerate the whole skin layer and generate human skin, and they also secrete paracrine signals. Human AFSCs accelerate skin wound closure by promoting re-epithelialization for up to 14 days.[20] Sun et al[16] reported that AFSCs can differentiate into keratinocytes in vitro, with a typical keratinocyte structure and an abundance of cytoplasmic tensinofibrils, and AFSCs express the typical keratinocyte markers — K5, K14, and K10 — in the three-dimensional (3D) gas-liquid culture layer. To test the proliferation of AFSCs, 1×10^5 CD117+ cells were isolated from 5 mL of AF after 1 month and amplified to $(6.4 \pm 2.3) \times 10^9$ cells after being sub-cultured 10 times, at the time when they maintained the characteristics of low-passage-number AFSCs. In a mouse model of skin defects, in which 1×10^6 AFSCs were injected into each 1 cm^2 wound and the AFSCs differentiated into highly proliferative keratinocytes, type I collagen decreased remarkably, and type III collagen increased in regenerated wounds. The wound closure rate and granulation tissue size were not affected.[41]

The therapeutic potential of stem cells is mediated by their ability to produce paracrine and exocrine factors, which promote stem cell proliferation, migration, differentiation, angiogenesis, and immunomodulation. Accordingly, AFSCs inhibit the expression of immunogenic and pro-inflammatory factors, such as IL-1β, IL-6, and TNF-α, and the costimulatory molecules B7H4, Cox-2, and Mac3. They also indirectly promote the secretion of bFGF, VEGF, CXCL12, TGF-β1, and insulin-like growth factor (IGF) through paracrine secretion. These conditions are conducive for the formation of a mildly inflamed wound microenvironment that benefits and promotes wound healing [Figure 3].[16]

Vascular regeneration and AFSCs

Blood vessel formation creates channels for rapid oxygen, nutrient, growth factor, and waste diffusion; conversely, reduced angiogenesis delays wound healing.[51] The repair factor VEGF induces the differentiation of AFSCs into functional endothelial-like cells; AFSC-derived endothelial cells are precursors of cells for the vascular lineage.[52]

Moreover, human AFSC-derived exosomes promote angiogenesis by upregulating the expression of VEGF
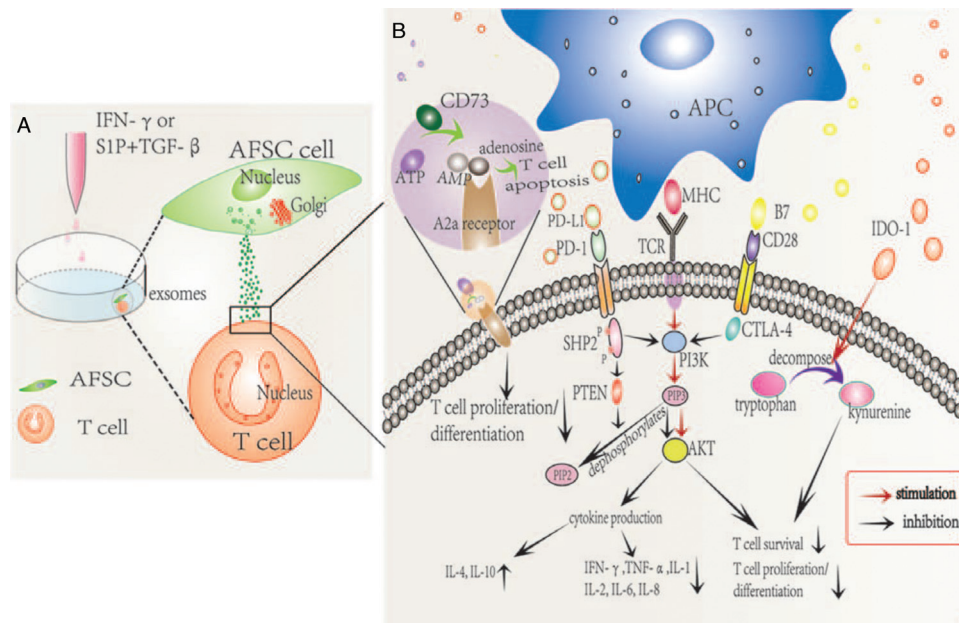
Figure 2: The immunomodulatory pathway of AFSCs. (A) Both S1P with TGF-β1 or IFN-γ cocultured with AFSCs can promote its immune regulation through the paracrine effect. (B) The CD73 on the membrane surface of exosomes, and IDO1, PD-1, and B7H4 contained in it participate in the immune regulation of T cells. AFSCs: Amniotic fluid stem cells; A2a: Adenosine A2a Receptor; AMP: Aadenosine-monophosphate; AKT: ATP-dependent tyrosine kinases; APC: Antigen presenting cells; ATP: adenosine-triphosphate; CTLA-4: Cytolytic T lymphocyte-associated antigen 4; IDO: Indoleamine 2,3-dioxygenase 1; IFN-γ: Interferon-γ; MHC: Major histocompatibility complex; PD-1: Programmed death 1; PD-L1: PD-ligand 1; PI3K: Phosphatidylinositol 3-kinase; PIP3: Phosphatidylinositol (3,4,5) -triphosphate; PIP2: Phosphatidylinositol (4,5) -triphosphate PTEN: Phosphatase and tensin homolog; S1P: Sphingosine-1-phosphate; SHP2: Protein tyrosine phosphatase; TGF-β: Transforming growth factor-β; TCR: T cell receptor.
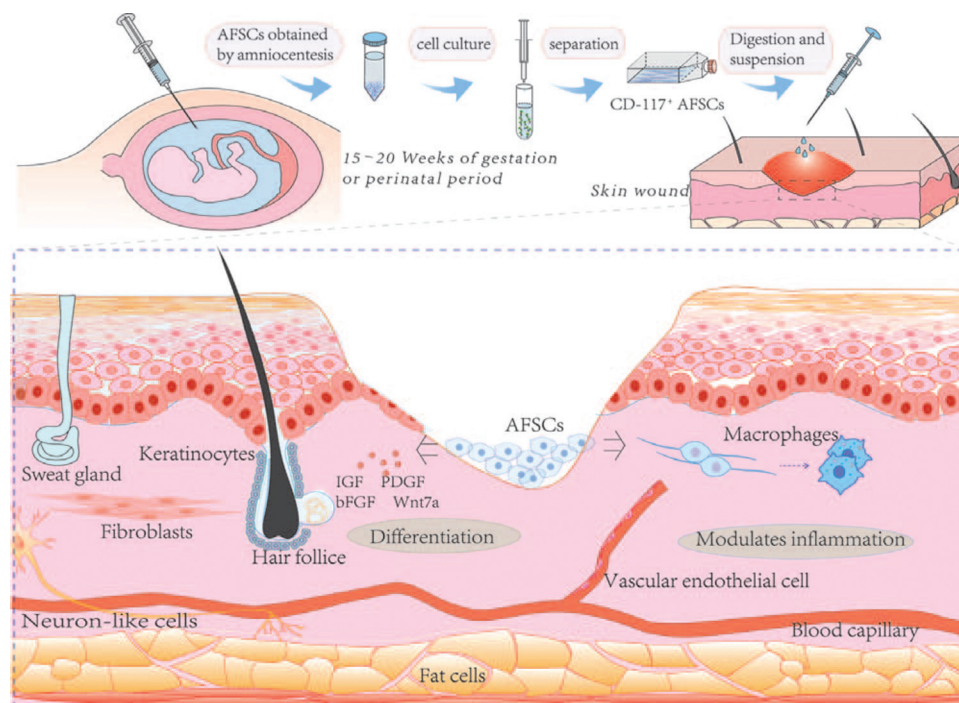
Figure 3: The role of AFSCs in wound healing. AFSCs can be injected into the full-thickness skin defect wound, regulate the local microenvironment and the inflammatory reaction through the paracrine pathway, and can also differentiate into various cells, which could improve the quality of wound healing. AFSCs: Amniotic fluid stem cells; bFGF: Fibroblast growth factor b; IGF: Insulin-like growth factor; PDGF: Platelet derived growth factor; Wnt: Wingless / Integrated.

allele (VEGFA) and platelet endothelial cell adhesion molecule 1 in endothelial cells, facilitating the formation of capillary-like networks.[52,53]

In a rat model of a full-thickness ischemic skin flap, AFSCs recruited CD31+/VEGFR2+ and CD31+/CD34+ cells into the ischemic subcutaneous tissue, induced endothelial

progenitor cells to initiate endogenous repair and form vascular endothelial cells, and improved local vascularization and blood supply to the damaged flaps.[53] Exosomes derived from AFSCs contain monocyte chemoattractant protein-1, IL-8, stromal cell-derived factor-1α, and VEGF, and are cytoprotective, pro-differentiative, and chemoattractive to endothelial cells.[54,55] When cultured under hypoxia, AFSCs cause endothelial regeneration and angiogenesis, serving as a promising source of stem cells with considerable endothelial regenerative potential.[56]

Skin appendage regeneration and AFSCs

Epidermal stem cells from hair follicles (HFs) around a wound can migrate to a wound site and promote reepithelialization to facilitate healing. The functional unit of hair includes the sebaceous gland and the arrector pili muscle that regulates temperature and perspiration. Paracrine signaling molecules produced by AFSCs, such as IGF, bFGF, Wingless/Integrated (Wnt)-7a, and platelet-derived growth factor, promote hair growth through the telogen-to-anagen transition in HF, an increase in HF density, and an increased rate of HF regeneration.[23] Concurrently, new HFs in mice secrete bone morphogenetic proteins (BMPs), which are growth factors that can transform fibroblasts into adipocytes and significantly reduce scar formation.[57]

Sweat gland (SG) cells play a vital role in thermoregulation and skin metabolism and have been a major focus of wound healing and skin appendage regeneration. Liang et al[58] induced CD117^{+} human AFSC differentiation into sweat gland-like (human AFS-SG) cells using conditioned media collected from human SG cells. Among them, sonic hedgehog plays a role in the formation of SG tubular structure, which is located downstream of the ectodermal dysplasia antigen (EDA) / the EDA receptor (EDAR) pathway and regulates the formation of secretory regions,[59] and epidermal growth factor (EGF) can improve the differentiation efficiency of AFS-SG cells. Human AFS-SG cells are similar to human submandibular gland cells. Under transmission electron microscopy, the typical cellular structure/microvilli of SG cells can be seen expressing SG-related markers: EDA, EDAR, keratin-8, and carcinoembryonic antigen. Human AFS-SG cells express acetylcholine receptor (M3R) and can form SG tubular structure under 3D culture, which has the potential to respond to acetylcholine in a way that is similar to the response of normal SGs.[58]

At present, there is no report on the successful induction of HF regeneration by AFSCs, yet studies have shown that stem cells can trigger the differential expression of the Wnt signal in the epidermis and dermis through the antagonistic effect of BMPs and sonic hedgehog in the same individual and control whether the stem cells will be involved in SG or hair regeneration.[60]

Peripheral nerve injury repair

Human AFSCs secrete various neurotrophic factors required to generate nerves, such as neurotrophic factor 3, brain-derived neurotrophic factor, ciliary neurotrophic factor, and glial cell line-derived neurotrophic factor.[61] In miniature pigs, AFSCs can accelerate the regeneration of nerve fibers, promote the growth of injured nerves across the nerve gap, and improve motor nerve conduction velocity. It is possible that AFSCs can be induced to differentiate into Schwann cells and participate in the regeneration of an injured sciatic nerve, promoting the functional recovery of injured nerve tissue through neurotrophic factor secretion or interaction with remaining Schwann cells, axons, or immune cells.[62] Using human AFSCs to treat myelomeningocele fetal rats causes stem cells to migrate to the lesion site and secrete growth factors to inhibit neuronal injury and induce neurogenesis [Figure 4].[63]

Application of AFSCs in tissue engineering

Although AFSCs have great potential in the process of wound healing, various challenges remain. For example, the survival time of allogeneic AFSCs in vivo is short, and how to control the differentiation direction and paracrine pathway of AFSCs in vivo is not currently understood.[64]

To improve the survival rate of AFSCs, maintain their biological characteristics, and promote wound healing, a polymer resembling the human fibrin matrix has been developed, which transports nutrients and stem cells to wounds. In this method, the AFSCs were cross-linked with a heparin-conjugated hyaluronic acid hydrogel to prolong AFSC paracrine signals, improve re-epithelialization and wound closure, and increase angiogenesis and ECM synthesis in vivo.[65] Different polymers have specific physicochemical characteristics and functional groups that allow for the controlled synthesis of biomaterials with desired properties for a wide range of therapeutic wound-based applications. Both natural and synthetic polymers are currently employed to treat skin wounds.[64,66] When used with biomaterials, c-kit[+] AFSCs at passage four plated at 3000 cells/cm^2 on gelatin-coated plates can generate endothelial cells and vascular pericytes, form capillary-like networks in fibrin/polyethylene glycol hydrogels, and induce angiogenesis in situ (when injected into hydrogels).[67] Lloyd-Griffith et al[68] created a vascular system in vitro using AFSCs co-cultured with human umbilical vein endothelial cells (HUVECs) on a collagen-chondroitin sulfate scaffold; 2×10^5 AFSCs and 3×10^5 HUVECs were seeded onto each side of the scaffold in 2 mL of standard EndoGro[TM] endothelial cell media (Millipore, MA, USA). The AFSCs acted as pericytes and interacted with HUVECs to form a micro-capillary-like structure. Weber et al[69] created vascular grafts from tubular vascular biocompatible scaffolds using AFSCs in a mobile bioreactor system. However, the polymer cannot provide the morphological structure of the epidermis, dermis layer, and subcutaneous tissue completely. It is impossible to achieve a uniform distribution of skin appendages under a single induction condition or completely reproduce the multilayer structure of the skin. Thus, polymers cannot completely mimic the function and anatomy of natural human skin.

Tri-layered skin constructs have been developed based on detailed studies of stem cell therapy and the emergence of
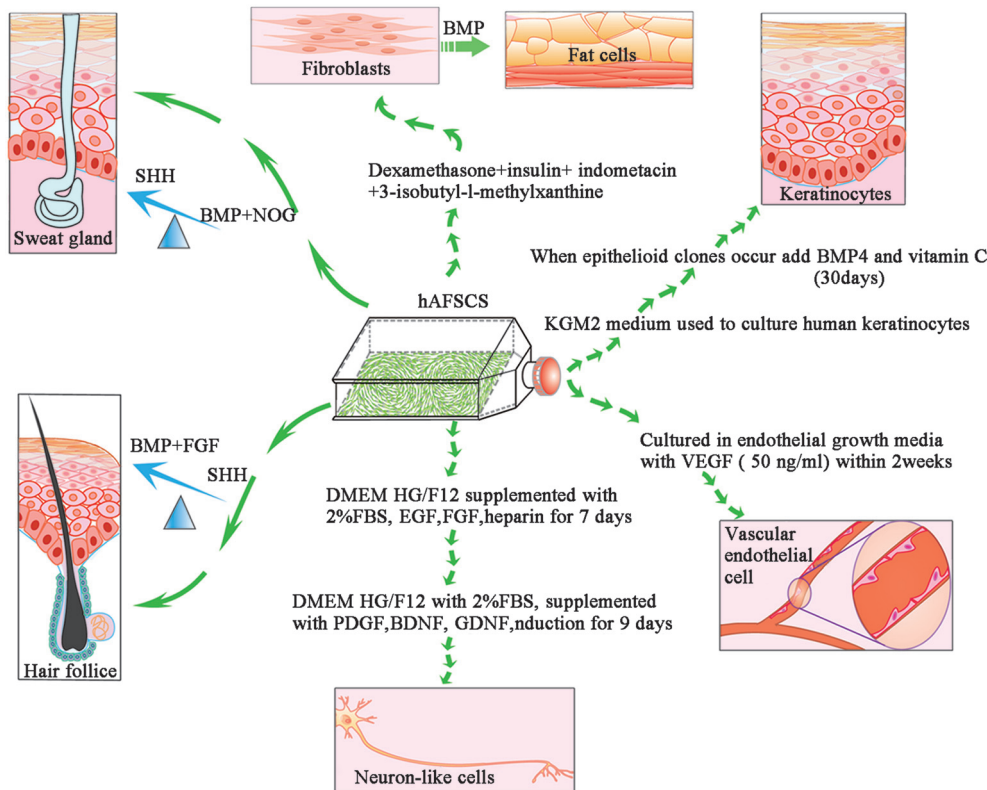
Figure 4: Transformation conditions of AFSCs. Within appropriate conditions, AFSCs can differentiate into fibroblasts, keratinocytes, vascular endothelial cells, and neuron-like cells. The presence of BMPs and SHH regulate the differentiation of AFSCs into HF or SG cells. AFSCs: Amniotic fluid stem cells; BDNF: brain-derived neurotrophic factor; BMP: Bone morphogenetic protein; DMEM HG/F12: Dulbecco's modified Eagle medium high glucose or F12 medium; EGF: Epidermal Growth Factor; FBS: fetal bovine serum; FGF: fibroblast growth factor; GDNF: glial cell line-derived neurotrophic factor; HF: Hair follicle; NOG: Noggin; PDGF: Platelet derived growth factor; SHH: Sonic HedgeHog; SG: Sweat gland; VEGF: Vascular endothelial growth factor.

nanotechnology and 3D bioprinting technologies.[66] These constructs include hypodermal adipose tissue, along with adequately vascularized dermis and epidermis, and together they provide the closest mimic to human skin for full-thickness wounds. A fibrin-based tri-layered skin construct has been synthesized by depositing adipose-derived stem cells, fibroblasts, and keratinocytes (which can be differentiated from implanted AFSCs) in the fibrin matrix to mimic the hypodermis, dermis, and epidermis, respectively.[70] A gas–liquid skin tissue 3D culture system has also been used to promote AFSC differentiation into keratinocytes and create a simulated functional epidermis on a matrix of fibroblasts and collagen.[71]

Although AFSCs are potentially powerful tools in aiding wound regeneration, current skin tissue engineering techniques cannot fully simulate the complex structure of natural skin, making restoration of normal skin function difficult. Thus, suitable polymer scaffolds must be developed to help AFSCs adapt to different extracellular environments and control stem cell differentiation.

Conclusion

The past decade has seen extensive investigation into the biological characteristics of AFSCs. The potential of AFSCs in wound regeneration has paved the way for their use in a range of clinical applications. AFSCs have strong self-replication ability, are safe, easily accessible, and capable of yielding large numbers of cells without provoking ethical concerns. They have low immunogenicity and do not undergo teratoma formation, making them suitable for cell transplantation from the same genus or from different individuals. They are an ideal source of stem cells.

There are two views of the AFSC mechanisms of wound healing. One is that stem cells enter the body and directly differentiate into the cells needed to regenerate injured tissue and that they act as paracrine effectors in the wound microenvironment to promote regeneration. The other view is that AFSCs survive only up to 7 days after being injected into the wound.[72] Supporters of this view believe that AFSCs do not directly differentiate into the cells required for wound healing, but those transplanted into the wound still promote wound healing. This view emphasizes that stem cells regulate the wound microenvironment, alleviate inflammation, promote the migration of endogenous stem cells to the wound surface, and promote the dedifferentiation of adjacent adult cells into stem cells to regenerate wound.[73,74] At present, there are no clear methods for determining the fate of AFSCs after entering the body. However, both views agree that AFSCs are paracrine effectors that regulate the local microenvironment and promote wound healing.

Although AFSCs play important roles in wound treatment, they contain cell subsets with different functions.

How to accurately separate these subsets is conducive to make full use of their different functions. At the same time, they function differently in various microenvironments, which is important while considering their potential therapeutic applications. Treatment needs should be fully considered for the induction of AFSCs according to each microenvironment before clinical use to maximize the unique features of AFSCs. From this viewpoint, specific AFSC surface markers should be identified, and the conditions for their clinical use should be explored. Determination of the conditions for obtaining a stable cell system with an adequate amount of the appropriate cytokines required for wound regeneration also requires further investigation.

Funding

Conflicts of interest

None.

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