Artykuł przeglądowy *Review*

Stem cells in wound healing

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Summary

The skin is a complex structure performing many essential functions in the body. Their external localisation makes them vulnerable to damage, which impairs their protective functions, which could cause local and systemic infections, excessive water loss and many other dangerous issues. Because of this, the problem of wound management affects both human and veterinary medicine. Selecting an appropriate therapy dedicated to a specific type of skin injury is a significant challenge for clinicians and researchers. Owing to the multi-phase and multifactorial nature of the wound healing process, contemporary regenerative medicine is striving to assist this process on several levels. In recent years, there has been rapid development of cell-based therapies in this field, including the use of stem cells for treating wounds in veterinary patients. The latest research and reports demonstrate that mesenchymal stem cells (MSCs) exhibit the greatest potential; these cells may be sourced from adipose tissue, bone marrow, or the umbilical cord. Studies have shown that the use of MSCs, whether autologous or allogeneic, accelerates the healing process by enhancing cell proliferation, angiogenesis, collagen production, and modulation of the inflammatory response. MSCs also exhibit beneficial paracrine effects, stimulating the recruitment of cells involved in tissue repair and proper re-epithelialisation, as confirmed by histopathological and molecular analyses. To date, cell-based therapies have been successfully applied in the treatment of both acute and chronic wounds, mainly in dogs and horses, enabling appropriate regeneration of skin in cases that previously proved resistant to conventional therapies. Research results suggest high efficacy and safety of MSCs in wound treatment; however, there is some information regarding the potential for teratoma development in connection with such therapies. Therefore, further work is required to standardise protocols and optimise the dosages and administration routes for the cells. Stem cell therapies represent an innovative and promising alternative to traditional wound treatments in veterinary practice and may potentially pave the way for new solutions in human patient care. This article constitutes a compendium of the currently available knowledge regarding the acquisition and application of stem cells in contemporary wound therapy.

Keywords: wound healing, tissue engineering, cell lines, difficult-to-treat wounds

The skin, the body's largest organ, is a complex structure performing many essential functions in the body. Anatomically, the skin consists of three main layers: the epidermis (outermost, made mostly of keratinocytes arranged in four layers, with stem cells in the stratum basale), the dermis (containing a papillary and reticular zone, rich in connective tissue, various cells, blood and lymphatic vessels, and glands), and the subcutaneous tissue (primarily loose connective and fat tissue) (55). The skin is responsible for creating a barrier that protects the body from the influence of many physical, chemical, and biological external factors. Another important function of the skin is to protect the body from water loss. It is also a key organ involved in the process of thermoregulation and vitamin D synthesis. Since the skin has direct contact with the environment, it is one of the most important sensory organs, responsible for receiving many stimuli (8, 55).

The external localisation of the skin makes it vulnerable to damage, which impairs its protective functions (97). Wounds and burns create entry points for pathogens, increasing the risk of local and systemic infections, water loss, and potentially septic shock (91). Especially dangerous are chronic wounds in the course of which the healing processes are not proceeding normally. In these cases, intensive effective therapy is necessary to save the health and life of patients (97). Developing better treatments for severe skin damage, including stem cell therapies, is a key challenge in modern medicine (30, 33, 91, 97).

Wound healing proceeds in four phases: haemostasis (blood clot formation to prevent blood loss), inflammation (recruitment of immune cells to eliminate debris and pathogens), proliferation (fibroblasts and keratinocytes fill the wound and form new tissue, including blood vessels), and remodelling (reorganisation of the extracellular matrix and collagen synthesis, resulting in scar formation and partial functional restoration) (11, 26). Wound healing is influenced by numerous factors, such as general health, chronic diseases (e.g., diabetes, circulatory disorders), poor diet, addictions, wound type, infection, and improper care (10, 20). Delays in healing can result in chronic wounds, which often stall in the inflammatory phase and present serious medical challenges due to pain, infection risk, loss of function, and possible amputation. Improving the healing of chronic wounds remains a major medical and research priority (13, 20).

As research shows, stem cells therapies are currently among the most widely studied medical procedures in wound healing (34). Most studies indicate that the best results are achieved using multipotent mesenchymal stem cells (MSCs).

There is ongoing debate among researchers regarding the appropriate nomenclature for a certain cell population: mesenchymal stem cells (MSCs) and mesenchymal stromal cells (MSCs). The main difference between these cells lies in their differentiation potential. Mesenchymal stem cells represent a population of stem cells with proven progenitor functionality, including self-renewal and differentiation capabilities. In contrast, mesenchymal stromal cells constitute a collective population of cells possessing significant secretory, immunomodulatory, and homing properties (99).

To standardise the nomenclature and enable comparison of results obtained by researchers, the Mesenchymal Stem Cells and Tissue Stem Cells Committee of the International Society for Cell Therapy (ISCT's MSC Committee) proposed minimal criteria for defining human and various mammalian MSCs. Firstly, these cells must be plastic-adherent. Secondly, they must express the surface positive markers typical of MSCs: CD73, CD90, and CD105, and lack expression of haematopoietic markers: CD34, CD45, CD14 (or CD11b), CD79α (or CD19), and HLA-DR(18).

MSCs are mostly derived from adipose tissue (ASCs) and bone marrow (BMSCs). In the treatment of chronic wounds are also using stem cells obtained from the umbilical cord (UCSCs). But compared to other types of MSCs are used less frequently (34, 68). In recent years, the importance of induced pluripotent stem cells (iPSCs) has also been steadily growing. This cells are obtaining in *in vitro* conditions from mature cells such as fibroblasts, keratinocytes, and blood nucleated cells, utilising genetic engineering methods. iPSCs are characterised by many features which are similar to this belonging to embryo deriving stem cells. Because of this similarity, iPSCs could be an effective substitute for them. This aspect significantly reduces ethical controversies related to the use of embryonic stem cells (15, 83, 90).

Nevertheless, despite the aforementioned properties of MSCs, their clinical application in wound therapy may encounter certain limitations. The wound microenvironment under inflammatory conditions is characterised by poor local vascularisation, nutrient deficiency, hypoxia, ischaemia, elevated levels of pro-inflammatory cytokines, and reactive oxygen species (ROS) (87, 101). Consequently, cells administered into such a hostile environment often experience a reduction in number due to anoikis induced by oxidative stress. Therefore, these cells face a significant challenge, which may substantially impair the modulation of their regenerative capacity and, consequently, reduce the overall efficacy of the therapy (101).

Since the literature data in this area remain ambiguous, the aim of the study is to evaluate the potential of various stem cells in promoting wound healing.

Stem cells

Stem cells are undifferentiated cells present in both embryonic and adult tissues. They are characterised by many features, such ability to self-renewal and differentiation into fully specialised body cells (22). This allows these cells to perform numerous important functions in the body at every stage of their development (108). During the embryonic period, stem cells are responsible for the formation of all body tissues and structures, enabling the development of a fully functional organism (79). In the postnatal period, stem cells facilitate the growth and regeneration of damaged body structures (22, 108). The activity of these cells also differs in different parts of the body. The highest intensity of stem cells proliferation occurs in structures such as bone marrow and skin (22, 79, 108). In organs such as the exocrine part of the pancreas, division of stem cells is activated only in the case of degenerative diseases that require regeneration (108). It can be distinguished that the stages of stem cells specialisation vary in the range of cells that can be transformed (22, 79, 108).

Totipotent cells have both the highest capacity to self-replicate and ability to transform into all mature body cells. An example of totipotent cells are cells that make up the embryo in the first few days of its development. They are characterised by many specific molecular features such loss of DNA methylation occurring mostly in 5-positon of cytosine (5mC). Totipotent cells indicated also significant changes in prenucleus structures associated with differences in structure of histones in paternal chromatin (52, 59,

108). Pluripotent cells can transform into all body cells except trophoblast cells. In the molecular aspect pluripotent stem cells are characterised by intensive expression of the NANOG, OCT4 and SOX genes (7, 92, 96, 108). Both of these cells occur only during the embryonic period. The stem cells, which can be found in mature body tissue, are multipotent and unipotent cells (52, 92). Multipotent cells can only transform into a few particular types of cells, e.g. bone marrow stem cells and hair follicle stem cells (52). Unipotent cells can transform only into one type of cell; they can include cells such as epidermal stem cells and satellite cells in skeletal muscle tissue (22, 108).

Collection and isolation of adipose tissue cells (ASCs)

Adipose-derived stem cells (ASCs) are widely used in wound healing due to their easy, minimally invasive collection from lipoaspirate or subcutaneous fat (24, 88). Samples must be stored under sterile conditions and transported to the laboratory as quickly as possible; they may be kept at room temperature for no longer than 24 hours (103). The tissue is centrifuged, after which only the fat layer is retained to ensure optimal cell recovery. The material is then washed several times with phosphate-buffered saline (PBS) containing penicillin/streptomycin to eliminate contaminants, blood residues, and remnants of tumescent solution (2, 103). In the next stage, enzymatic digestion of the tissue is performed by the addition of a collagenase type I solution (17, 24, 36, 39). The mixture is incubated for 30 to 120 minutes at 37°C with 5% CO_2 under continuous agitation at 500 rpm, subsequently neutralized with serum, and centrifuged at 3,000 rpm for 5 minutes at 4°C (39). The resulting pellet is resuspended in a red blood cell lysis buffer and incubated on ice for 2-10 minutes (24, 39). The cells are then washed with complete culture medium (e.g. MEM α, DMEM) or PBS supplemented with 2% antibiotics, and centrifuged again to remove undigested tissue fragments and isolate the stromal vascular fraction (SVF). The final pellet is resuspended in complete culture medium, passed sequentially through 70-100 µm meshes, and centrifuged once more (24, 103). It is important to note that the SVF is a heterogeneous, versatile cellular system. Therefore, to optimise the use of SVF in regenerative therapy and assess its clinical potential, SVF profiling is performed. This includes analysing the expression of cell surface markers (CD31 – endothelial cells, CD34 – progenitor cells, CD90 – mesenchymal stem cells, CD45 – haematopoietic cells) and the functional properties of these cells, which help in determining their composition, regenerative, immunomodulatory, and angiogenic capacities (24, 36, 93, 103). The cells thus obtained are suspended in culture medium. The cultures are incubated at 37°C with 5% CO_2 (24, 36, 39, 103). After 24-72 hours, once the cells have adhered to the plastic, the medium is aspirated and the cells are washed twice with warm PBS to remove non-adherent cells. The fibroblast-like cells are then incubated until they reach 70-90% confluence, which typically occurs within 7-10 days (36, 39, 98, 103). ASCs are more readily available in large quantities compared to bone marrow stem cells, making them easier to obtain for therapeutic use (2, 24, 39).

Autologous stem cell transplants are typically preferred due to their lower rejection risk. However, in diabetes, donor stem cells may be more effective than patient-derived cells, likely due to cell damage from high activity of ROS (34, 53). Studies also show that the mixed-cell population in SVF often yields better clinical outcomes than isolated ASCs alone. This is due to presence of many cells other cells in SVF than in pure ASCs solution. These cells are responsible for secretion of many factors which have synergic action with substances releasing by ASCs (29, 38).

Collection and isolation of bone marrow stem cells (BMSCs)

Bone marrow stem cells (BMSCs), known as non-haematopoietic stem cells (HSCs) that are located in the medullary stroma of bone marrow, are utilised in wound healing. However, their acquisition is more challenging compared to adipose-derived stem cells (ASCs). They are usually aspirated from marrow-rich bones (e.g., iliac crest, femur) using a syringe with an anticoagulant, e.g. heparin (71). Samples should be transported to the laboratory within 30-120 minutes, at room temperature (20-23°C), to retain cell viability (23). The marrow is filtered through a 70-100 µm mesh to remove bone fragments, cell clusters and fat, diluted 1 : 1 or 1 : 3 in PBS, and separated by Lymphoprep or Ficoll-Paque density gradient centrifugation at 20-23°C to isolate mononuclear cells (21). After removal of the supernatant, the isolated mononuclear cells are resuspended in standard BMSC isolation medium like DMEM or MEM α containing 2-10% foetal bovine serum. These are cultured under standard conditions (37°C, 5% CO_2), with non-adherent cells removed and media changed every 2-3 days (21, 23, 41).

Once cultures reach about 80% confluence, BMSCs are ready for expansion or therapeutic use (23). BMSCs can also be obtained from peripheral blood after stimulating their release with granulocyte colony stimulating factor (G-CSF) (105). Blood is then collected, and stem cells are isolated, often followed by *in vitro* expansion to achieve a sufficient cell concentration for effective therapy, thus enhancing their clinical impact (86). BMSCs *in vitro* exhibit adhesion to polystyrene and express surface molecules CD105, CD73, and CD90 (32).

Collection and isolation of umbilical cordstem cells (UCSCs)

Stem cells can be isolated from both umbilical cord blood and the umbilical cord tissue itself, such as Wharton's jelly and the cord lining. The umbilical cord is collected aseptically from healthy donors, either from caesarean sections or natural deliveries at 38-40 weeks of gestation in humans (42, 94).

Umbilical cord blood is collected from the umbilical vein after birth with an anticoagulant (111). For optimal cell viability (~90%), blood must be processed within 24 hours (69). In the lab, mononuclear cells are isolated by dilution and density gradient centrifugation, then washed and plated in culture media (69, 111). Fibroblast-like cells emerge in 7-14 days and are expanded when cultures reach ~80% confluence (69, 107, 111).

In the case of umbilical cord tissue, it is immersed in sterile containers filled with a transport medium, such as PBS or DMEM/F12 supplemented with antibiotics and an antifungal agent (42, 45).The tissue is transported at 4°C and should be processed within 12 hours of collection. The umbilical cord is cleaned, cut into segments, and the blood vessels are removed, keeping only Wharton's jelly (45). Tissue is enzymatically digested, filtered, washed, and centrifuged (74, 81). The resulting cells are cultured in cell growth medium with supplements at 37°C, with medium changes every 24 hours, until reaching 70-80% confluence (94).

In the explant (outgrowth) method, fragmented Wharton's jelly (without blood vessels) is placed in culture flasks with growth medium (42, 45). After about seven days, cells migrate out and form a monolayer. Tissue fragments are then removed, the medium is changed every 2-3 days, and cells are passaged at 70-80% confluency, typically after 10-14 days (40, 45). Cultures are maintained up to 2-3 passages for further use (45).

Obtaining of the induced pluripotent cells (iPSCs)

The method responsible for obtaining iPSCs has been developed in 2006 year by Japanese research team leading by Shinya Yamanaka (90). The process of obtaining iPSCs begins with harvesting mature cells from patient tissues. The most often utilising cells in this procedure are fibroblasts, keratinocytes and nucleated blood cells. They are also several studies showing that cells isolated from teeth pulp could be useful in obtaining iPSCs. The second step of this process is insert the four particular exogenous gens into the somatic cell genome by using natural vectors in most cases. Most often, retroviruses containing the above-mentioned genes in their genome are used for this purpose. After delivery into the nucleus of the somatic cells, gens OCT-3/4, SOX2, KLF4 and c-MYC integrate witch their DNA and secondly the they are expressed. The effects of this activity are bioactive factors which cause the changes in metabolical pathways of somatic cells leading to transformation into the stem cells. Besides the method including utilising of the viral vectors researchers developed several other procedures enable to introduce this gens into the cellular genome. One of them is the method using episomal vectors which do not integrate with cellular genome and can be removed after the procedure of cell transformation. But they enable the full expression of the genes introduced into the cell using them (15, 83).

Use of stem cells in wound therapy

Stem cells can be delivered to wounds locally (e.g., injections near the wound or in scaffold dressings) or systemically (intravenous or intramuscular injection) (58). Biomaterials made from natural or synthetic polymers help maintain cell viability and promote healing (53). Both local and systemic applications accelerate wound healing and are promising for chronic wound treatment (50, 60).

Numerous studies have shown the positive impact of various types of MSCs on wound healing (30). During these tests, they also tried to determine the direct mechanism responsible for the promising properties of these cells. Stem cells can enhance healing in various ways (17, 30). Many studies indicate that the most important aspect of stem cells activity in a wound environment is the release of numerous cytokines that accelerate the regeneration process of damaged tissues (30, 56). A crucial aspect of using stem cells in wound treatment is their ability to differentiate into various mature cell types (56). Properly introduced into a wound, stem cells can differentiate into cells that directly participate in filling skin tissue loss, such as fibroblasts (30, 56). They have also demonstrated angiogenic activity, which enhances the spread of blood vessels in the area of the wound. The presence of these cells also has a stimulating impact on the extracellular matrix deposition (17, 30).

Each stage of wound healing and skin regeneration is supported and coordinated at multiple levels by stem cells (84). As a result of chemotaxis, MSCs accumulate in the area of damaged tissue and participate in its repair (30). Stem cells exhibit anti-inflammatory effects by limiting inflammation at the wound site and influencing macrophage polarisation towards the M2 (anti-inflammatory) phenotype. They also possess immunomodulatory capacities by modulating the differentiation of T lymphocytes into Th2 and regulatory T cells, which alleviates inflammation and promotes the transition to the proliferative phase (84). additionally affect natural killer cells, dendritic cells, and neutrophils, modulating their activity in favour of reparative processes (73).

Through paracrine activity, stem cells produce and release bioactive molecules such as growth factors,

cytokines, and chemokines. Key growth factors include epidermal growth factor (EGF), fibroblast growth factor 2 (FGF2), hepatocyte growth factor (HGF), insulin-like growth factor-1 (IGF-1), keratinocyte growth factor (KGF), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and transforming growth factors alpha and beta (TGF-α and TGF-β) (73, 84). Pro-inflammatory cytokines released by MSCs include interleukin-1 alpha and beta (IL-1α, IL-1β) and interleukin-6 (IL-6); anti-inflammatory cytokines include prostaglandin E2 (PGE2) and interleukin-4 (IL-1, IL-4); proliferative cytokines include IL-6 and interleukin-10 (IL-10) (73, 84, 93). Chemokines released by stem cells that participate in wound healing include interleukin-8 (IL-8), monocyte chemotactic protein-1 (MCP-1), macrophage inflammatory protein-1 (MIP-1), and stromal cell-derived factor-1 (SDF-1) (29, 73, 84, 88).

These bioactive molecules modulate the activity of cells involved in wound healing by enhancing angiogenesis and collagen synthesis, supporting reconstruction of the extracellular matrix, which accelerates wound closure and provides optimal conditions for tissue regeneration and remodelling (29, 35, 43). Additionally, MSCs reduce bacterial load in the wound by enhancing neutrophil migration and phagocytic activity through the secretion of factors such as granulocyte-macrophage colony-stimulating factor (GM-CSF) (84). The action of MSCs decreases scar formation propensity, reduces fibrosis, and accelerates re-epithelialization by influencing the composition and remodelling of the extracellular matrix, thereby improving the quality and speed of wound healing (73).

Adipose tissue stem cells in wound healing

The most important factors released by ASCs with a significant impact on the processes occurring in the chronic wound environment include basic fibroblast growth factor (bFGF), EGF, HGF, insulin-like growth factor-binding protein 6 (IGFBP-6), TGF-β and VEGF. They stimulate the migration and proliferation of fibroblasts, keratinocytes, and vascular epithelial cells by activating the phosphoinositide 3/protein kinase B(PI3K/Akt) orfocal adhesion kinase/extracellular signal-regulated kinase (FAK/ERK1/2) pathways (77, 78). Some authors associate the activity of these factors with an increased vascularisation rate and extracellular matrix synthesis. Park et al. (78) confirmed that bioactive substances released by ASCs are important and effective tools which enhance the regeneration of damaged skin tissue. The authors also propose that the influence of paracrine factors released by ASCs may have a greater impact on wound healing than the proliferation and differentiation of stem cells. They likewise indicated that secretome (solution containing substances obtained from ASCs) had a significantly greater impact on wound healing time than secretome obtained from human fibroblasts (78). Kim et al. (51) also found that growth factors released by ASCs significantly accelerate fibroblast proliferation and the synthesis of collagen fibres, an important component of the extracellular matrix. All of these processes significantly accelerate wound closure (51). Maharlooei et al. demonstrated that the administration of ASCs to a wound accelerates wound healing, but they found no correlation between the presence of ASCs and an increase in the rate of vascularisation and the number and density of collagen fibres (65). Despite this, Park et al. (78) indicated that secretory factors such as VEGF, bFGF, EGF, HGF and TGF-β1 released by ASCs stimulate collagen synthesis and development of blood vessels net in the area of wound. Like other researchers, they confirmed also that these substances increase fibroblast migration into damaged skin tissue (78). Kuo et al. (54) conducted a study demonstrating the effect of local ASCs injection on wound healing in rodents with diabetes, which is one of the most common causes of chronic wounds. The obtained results indicate that ASCs accelerate wound healing, which is associated with their release of multiple growth factors. However, Kuo et al. did not provide evidence of the ability of ASCs to differentiate into fibroblasts and other cells that make up skin tissue (54).

Many other studies have also confirmed the significant role of factors secreted by ASCs in biological regenerative processes occurring within wounds. Studies focusing on the exosomes released by stem cells have indicated that they contain numerous bioactive substances, such as tumour necrosis factor alpha (TNF-α), macrophage colony-stimulating factor (MCSF), and retinol binding protein-4 (RBP-4), which are responsible for regulating the inflammatory processes in the wound by influencing the activity of white blood cells. The action of these compounds lead to inhibit the excessive inflammation in the wound environment which is one of the most important causes of impaired wound closure. It is mostly associated with decrease of interferon alfa (IFN- α) secretion (strong pro inflammatory factor) and aggregation capacity of M1 macrophages (57, 66, 88). Exosomes contains also high concentration of pro angiogenic factors such miRNA-125α and miRNA-31and VEGF. Of essential importance is activity of miRNA-31 which can inhibit the anti angiogenic gene HIF1. Enhancement of the vascularisation rate under influence of these factors leads to the improvement of delivery of many substances necessary for the reconstruction of damaged tissues. ASCs also possess ability to secrete tissue inhibitors of metalloproteinases (TIMPs) responsible for inhibition of the enzymes from matrix metalloproteinases group (MMPs). These enzymes are responsible for degenerating collagen fibers which are the main compound forming the extracellular matrix. TIMP is a factor preventing against excessive activity of the MMPs which could interfere with

effective wound healing (43, 66, 88). The exosomes also contain high amounts of growth factors such FGF and EGF responsible for the stimulation of migration and proliferation of the fibroblasts. They are also source of IGF-1 the activity of which is associated with the initiation of fibroblast migration proliferation and differentiation which significantly increases the rate of reepithelialisation. The other important growth factor which is delivered to the wound by exosomes deriving from ASCs is KGF, which is one of the most important factors that stimuli proliferation and differentiation of keratinocytes (57, 66).

The ability of ASCs to transform into mature cells, replacing defects in skin tissue, was further confirmed by Altman et al., who demonstrated that ASCs applied to a wound using a special scaffold transform into cells that contribute to the formation of skin tissue structure (3). Trottier et al., using an *in vitro* model, also demonstrated that ASCs can transform into fibroblasts and other skin cells (98). There are studies which confirmed that ASCs can transform into endothelial cells developing in the wound area blood vessels (66).

Despite the above data, most researchers maintain that the paracrine factors secreted by ASCs play a key role in stimulating wound healing. However, most available research indicates that the use of ASCs in almost all cases leads to accelerated healing (3, 16). Furthermore, wounds treated with ASCs also show a reduced tendency to develop visible scars and discolouration (3, 5, 16).

Bone marrow stem cells in wound healing

BMSCs are also frequently used in chronic wound healing. They also participate in wound healing in physiological conditions without external application. It has been shown that in the case of skin injury, stem cells leave the bone marrow and accumulate around the wound (105). Many researchers indicated that after intravenous application, BMSCs were directed to areas with the greatest demand for regenerative factors (1). Like ASCs, BMSCs stimulate the regeneration of damaged tissues, primarily by releasing numerous factors that stimulate fibroblast and keratinocyte proliferation, as well as angiogenesis. The most important regenerative factors include vascular endothelial growth factor A (VEGF-A), EGF, erythropoietin, and stromal cell-derived factor-1α (SDF-1α). The ability of the stem cells to transform into other skin cells seems to be of significantly lower importance (16).

Similarly, researchers have widely studied the possibility of using BMSCs and exosomes obtained from them in clinical procedures to improve wound healing, just as they have with ASCs. Wang et. al conducted research which indicated that utilising BMSCs exosomes in combination with nuclear factor erythroid 2-related factor 2 (NFE2L2) and showed that these factors significantly improved wound closure process in diabetic animals (102). Positive correlation with using BMSCs exosomes and acceleration wound healing has also been confirmed by Jiang et al (46). Rodriguez-Menocal et al. (81) estimated the effect of the application of stem cells on radiation-induced chronic wounds in a mouse model. Researchers used three types of bone marrow preparations: isolated BMSCs, whole bone marrow, and cultured whole bone marrow. All of them accelerate the healing process. This study also showed that the application of whole bone marrow was the most effective factor in the aforementioned processes. Researchers believe that the higher concentration of cells in this particular concentrate may be linked to elevated levels of growth factors (compared to the other studied cell concentrates) and other cytokines, which play a role in the healing process (81). Similar results have been obtained by Ichioka et al. (44). They indicated that using stem cells in the wound environment accelerates the vascularisation process, which is one of the most important factors influencing the healing period (44). The presence of bone marrow cells in wounds also has a positive impact on the synthesis of the extracellular matrix, primarily collagenous proteins, as demonstrated by Fathke et al. (31). Some studies have also been conducted in human models. Research by Badiavas et al. (9) focused on the direct application of BMSCs in the treatment of chronic, non-healing wounds in patients who had previously received conventional treatment for over a year. In every case, the application of the cells resulted in wound closure. Similar positive outcomes were also confirmed by Dash et al. (19), who found that applying cells to a wound using a standard dressing significantly improved healing and reduced disease-related pain. Rogers et al. (82) used a bone marrow-derived cell concentrate containing stem cells to treat chronic ulcers resistant to standard treatment, and the local application of these cells stimulated and accelerated wound healing.

Gupta et al. indicated that utilising the autologous bone marrow aspirate on chronic wounds to significantly decreased the wound area in comparison to the control groups (37). On the other hand, Bonora et al. (12) conducted a study in which the researchers tried to estimate the effect of a preparation stimulating the proliferation and release of the patient's BMSCs into his bloodstream (12). Unfortunately, the results of this study were unsatisfactory. No significant changes were found in the healing process of chronic wounds between the study and control groups (12). The safety of novel medicals to topical application on the wounds has been checked by Andersen et al. (6). The data indicated that these medicinal products did not cause any undesirable effects in the treated patients. However, owing to the very small size of the study group (only two individuals), more detailed investigations on a larger patient cohort are required (6).

Umbilical cord stem cells (UCSCs) in wound healing

Umbilical cord stem cells (UCSCs) are mainly used in human medicine and are less commonly utilised compared to other stem cell types due to the difficulties in collecting and storing them, as they must be cryopreserved immediately after birth. Most parents opt not to do this, limiting access to these cells in adulthood. However, UCSCs can be used to treat chronic wounds, acting on skin tissue similarly to other stem cell types (30).

Numerous studies have demonstrated the impact of these cells on the wound healing process. UCSCs administered both locally using special scaffolds and peripherally via intravenous injection in all cases enhanced the reepithelialisation, revascularisation, and synthesis of extracellular matrix in the area of chronic diabetic ulcers in mice (110). Palma et al. (76) also indicated that transplantation of the UCSCs leads to an increase in revascularisation, which is associated with the acceleration of wound healing. The use of exosomes obtained from UCSCs cultures containing paracrine factors improves the regeneration of matrix and promotes the proliferation of skin cells in damaged tissue (111). Moreover, wounds treated by UCSCs showed a lesser tendency to form permanent, visible scars (110). Tan et al. (95) also confirmed positive properties of the bioactive substances releasing by UCSCs in wound healing. Similar to the previous study, secretom (a solution containing substances secreted by stem cells through exosomes) obtained from cell cultures were found to decrease the period of chronic ulcer closure by stimulating the growth of new blood vessels, the synthesis of extracellular matrix and increasing the proliferation of fibroblasts (62). Researchers claimed that this process is the result of the presence of various bioactive factors in the studies of secretom, including VEGF, FGF, TGF and EGF (95). Luo et al. analysed the impact of the local application of UCSCs on wound healing in a mouse model (62). An interesting aspect of that study was the confirmed ability of the stem cells to transform into skin fibroblasts. Moreover, UCSCs were found to be able to regulate the production of IL-10, TGF-β1, IL-6, and thrombospondin-1 (TSP-1) through paracrine pathways in the animal wound model, which promoted healing (62).

Induced stem cells in wound healing (iPSCs)

iPSCS similarly to the other upper described types of stem cells also a showed positive impact on wound healing. Because of their ability to secrete such growth factors as FGF, VEGF, TNF-α and PDGF which enable the regulation of processes which take place in the wound environment and have the ability to transform into mature cells which help to replace damaged tissues iPSCS, have great importance in regenerative medicine (64). Researchers conducted many studies which clearly proved the upper mentioned properties of these cells. Yan et al. (106) and Zhang et al. (109) indicated that using micro vesicles secreted by iPSCs which have been derived into the tissues surrounding the wound by subcutaneous injections, significantly accelerate wounds closure. In rodents belonging to the group cured by solution containing iPSCs exosomes researchers noticed a greater increased the rate of vascularisation and migration of the keratinocytes than in the control groups. Substances included in exosomes also stimulate migration of the fibroblasts toward the wound and synthesis and maturation of the collagen (106, 109). The above described researches clearly showed that iPSCs are also a very effective tool in chronic wound therapy. Similarly as in the other types of stem cells the greatest importance in this process is factors released by these cells which have deep regulatory influence on many physiological processes occurring in wounds. But despite such promising results of the studies many scientists have some concerns about therapies based on utilising this type of stem cells. Most of these concerns are associated with the risk of the proven teratoma development on the base of iPSCs. Because of this fact ways of the safe using of the iPSCs need to be subjected to further researches (15, 35).

Application of stem cells in veterinary medicine

Numerous preclinical and clinical experiments have been conducted to evaluate the feasibility of applying stem cells in the treatment of chronic or delayed-healing wounds in humans (9, 19, 30, 82, 95). Extensive research has also been undertaken using animal models of impaired wound healing in mice (3, 31, 44, 51, 62, 76, 81, 98, 51) and rats (1, 54, 65, 111). The studies presented provide robust evidence that stem cell therapies, both in humans and animals, offer effective support in the healing of refractory skin injuries, indicating considerable translational potential for implementing this approach in veterinary medicine. This may yield substantial benefits in the context of anticipated routine clinical applications.

Slow or impaired wound healing is a common problem among veterinary clinicians. This process can lead to inadequate regeneration of damaged tissues and, ultimately, unsatisfactory treatment outcomes. For this reason, veterinarians are continually striving to implement innovative therapeutic methods, which has led to an increased interest in MSCs in veterinary medicine (67). Numerous studies conducted to date in veterinary patients as well as in experimental animals (goats, sheep, horses, dogs, rats, mice) have demonstrated the beneficial effects of MSCs therapy on wound healing (28, 89, 100, 104). Mesenchymal stem cells are increasingly being used in clinical studies and clinical practice for the treatment of hard-to-heal wounds in

veterinary patients, primarily dogs and horses (25, 72, 80). Research also demonstrates their effectiveness in the treatment of atopic dermatitis (AD) and pemphigus foliaceus in dogs (25, 81). MSCs therapies promote the formation of new blood vessels, increase collagen synthesis, and stimulate the migration and proliferation of many skin cells, such as fibroblasts, which resulting in faster wound closure and earlier development of healthy granulation tissue, ultimately lead to faster skin regeneration (27, 100). Studies conducted on animal models (mice and dogs) have shown that systemic therapy with activated MSCs combined with antibiotics led to a significant reduction in the number of drug-resistant bacteria, improved wound healing, and a decrease in inflammation and fibrotic changes (48). Despite all the benefits described, different routes of MSCs administration carry varying risks for the patient; for example, systemic administration may cause the cells to become trapped in the lungs or capillaries, potentially leading to embolisms. Additionally, around 90% of the administered cells are lost due to factors such as hypoxia, inflammation, or rejection, which necessitates the use of a very large number of MSCs, which may lead to the development of teratoma (67).

Animals, including rodents (rats, mice), rabbits, and pigs, are commonly used as models for comparative evaluation of wound healing (85). However, the results obtained can vary significantly depending on the animal species, influencing both the rate and quality of healing as well as the outcomes of individual therapies. The choice of an appropriate species is crucial for the translational relevance of the results to human or veterinary medicine (85). For example, due to the thinner skin layer, wounds in rodents primarily heal through wound contraction, which distinguishes them from dogs, horses, pigs, or rabbits, whose skin and healing structure are much closer to those of humans, where re-epithelialisation predominates. For this reason, wound healing based on contraction, as observed in experiments involving mice and rats, may not translate directly to this process in other species (63, 85). Additionally, unlike humans and larger animals, microvessels in mice form more rapidly but are smaller, and the process of neovascularisation exhibits distinct dynamics, highlighting interspecies differences in both the scale and timing of this process following potential MSCs therapies (49). Furthermore, cytokine profiles differ between species, which can alter the magnitude and duration of the inflammatory response and consequently affect the immunomodulatory effect of MSCs (70). It is also noteworthy that rodents typically form minimal hypertrophic scars, whereas larger animals may exhibit fibrous tissue overgrowth (75). Therefore, when planning or interpreting translational studies, it is essential to consider differences in healing rates, fibrosis tendencies, and immunological responses between species, as these factors influence both the evaluation of efficacy and safety of stem cell therapies in veterinary medicine.

Mesenchymal stem cells may become a genuinely novel alternative method to conventional adjunctive wound therapies due to their anti-inflammatory and regenerative potential. However, before this treatment can be adopted into routine veterinary practice, further studies are essential to address outstanding questions and uncertainties regarding the mechanisms of wound healing mediated by MSCs (100).

Limitations in the use of stem cells

Despite promising results in preclinical and clinical studies stem cells therapies also indicate some limitations. The greatest concerns stem from the increased probability of development some types of neoplasms in patients subjected to these therapies. The highest risk of tumor occurrence is associated with using of the iPSCs because of the presence in their genomes of the c-MYK gene which is considered a proto-oncogene. For this reason, it is necessary to develop a cell multiplication system that will decrease number of cells with the probability of tumour transformation (35). Another problem is the low survival rate of the cells applied on the wound. Because of this it seems to be necessary to develop new types of scaffolding which increase the survival rate of the cells in the wound environment. Moreover, using stem cells derived from other individuals increase the risk of rejection reaction, which can lead to the failure of the entire therapy. Problematic are also the high costs of these procedures. Which necessitates the overall development of the technologies which will make them more available for wider group of patients (35, 61).

In the studies with MSCs, it is necessary to ensure an adequate number of cells, which requires large-scale expansion and inevitably raises issues of MSCs senescence and subsequent alterations in gene expression. Another important consideration is the variable stability of stemness and differentiation capacity among MSCs isolated from different sources (112). Early-passage MSCs exhibit multipotency; however, their differentiation potential and function decline progressively with each subsequent passage (4). Cells at passages 1-2 demonstrate greater therapeutic efficacy and higher multipotency compared to those at passages 3-4, while continued expansion leads to a diminution of stemness and a reduction in therapeutic potential (47). It has been shown that serial passaging of MSCs during long-term culture can adversely affect the expression of stemness-related genes (112). MSCs have a limited lifespan *in vitro* and ultimately undergo senescence, characterised by irregular cell morphology and reduced proliferation rate (4). Senescence may manifest even at early passages, following a period of normal growth, culminating in growth arrest. This process is accompanied by telomere attrition, and impaired cellular

function, all of which diminish the cells' proliferative and differentiation capacities (4, 47). In *in vitro* studies, passages up to 5 are most commonly examined. For application purposes, low-passage cultures are recommended; however, no universal or standard passage number has been established. It is important to continuously monitor changes in cell metabolism or appearance (which may indicate cellular ageing), as these changes could disrupt or halt the process of skin regeneration (4, 14).

Conclusion

The use of stem cells in wound therapy presents a promising strategy in modern regenerative medicine. To date, studies have demonstrated that these cells not only accelerate the healing process but also enhance the quality of regenerated tissues, reduce scarring, and minimise the complications associated with chronic wounds. Their unique ability to differentiate and modulate the immune response has led to their increasing application in the development of advanced therapies and bioactive dressings. However, several challenges remain to be addressed, including the standardisation of therapeutic methods, assessment of long-term safety, and optimisation of cell sourcing. Further large-scale clinical studies are essential to fully evaluate the efficacy and safety of this form of treatment. The development of innovative technologies and interdisciplinary collaboration is crucial to achieving a breakthrough in managing hard-to-heal wounds using stem cell-based therapies.

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