Chapter

Application of Cell-Based Therapies in Veterinary Dermatology

Carolina Mesquita, Bruna Lopes, Patrícia Sousa, Mariana Branquinho, Ana Catarina Sousa, Ana Lúcia Luís, Rui Alvites and Ana Colette Maurício

Abstract

Stem cells have been extensively studied in the field of veterinary medicine due to their unique characteristics. The last are undifferentiated cells with self-renewal, anti-inflammatory, and immunomodulatory capacity. Mesenchymal stem cells (MSCs) are widely used due to its simple isolation and expansion, being collected from different sources such as adipose tissue, bone marrow, peripheral blood, and umbilical cord. For that reason, MSCs have been studied and used as innovative therapies in the treatment of several diseases, such as tendinitis, bone regeneration, osteoarthritis, neuromuscular diseases, heart diseases, respiratory diseases, kidney disorders, ophthalmology, oncology, and dermatology. Concerning dermatological problems, the number of skin diseases in animals has been increasing in recent years. Skin diseases may be related to genetic conditions, external aggressions, or immunological disorders. Many of these skin pathologies are chronic, reason why the animals are subjected to long-term therapies, which can have deleterious side effects. This review aims to highlight the importance of cell-based therapies, using MSCs from different origins and their secretome, in the field of veterinary dermatology and in immune-mediated diseases such as atopic dermatitis, furunculosis, anal vasculitis, and scar tissue regeneration. These approaches should be further explored, as they have revealed promising results in the search for novel therapies.

Keywords: cell-based therapy, mesenchymal stem cells, skin diseases, veterinary dermatology, wound healing

1. Introduction

In the last years, regenerative medicine has been developing in fields such as wound healing and skin regeneration. The skin acts as a protective barrier that isolates the body from harmful agents and injuries. In addition, the skin also contributes to homeostatic maintenance, regulating the body's temperature and internal integrity. Age, tumors development, congenital defects, and degenerative diseases are some

of the factors associated with difficulties in wound healing, reason why regenerative medicine can be very helpful in the achieving better results [1]. The skin can be frequently injured because of both chronic and acute wounds (burns, diabetic ulcers, and atopic dermatitis), and these patients experience mental, physical and health constrains that can lead to a huge socioeconomic burden [2]. Recently, MSCs started to be used as therapeutic agents capable of regenerating damaged tissues and organs [3]. For that reason, new cell-based therapies have received attention in both human and veterinary medicine. MSCs are multipotent cells that derive from the embryonic layer of the mesoderm. Besides, under the right *stimulus*, these cells can differentiate into different lineages, such as osteoblasts, myocytes, chondrocytes, among others [3–5]. MSCs are undifferentiated cells with specific characteristics such as self-renewal capacity, originating cells with identical characteristics, and the potential or ability to differentiate from cells in mature tissues, which gives them the ability to repair tissues and organs [6, 7]. There are numerous clinical studies demonstrating the therapeutic potential of MSCs in various fields of veterinary medicine [3]. Moreover, it is known that conventional treatments, based on medical drugs, are often associated with unwanted side effects due to the re-use of these drugs. Nevertheless, despite the capacity of MSCs in wound repair and cutaneous regeneration, there are some limitations, such as the heterogenicity in the delivery protocols, site of delivery, and the lack of information concerning MSCs functional properties and phenotype [2].

Several dermatological problems have a congenital origin and a chronic/recurring nature, forcing these animals to receive repeated and prolonged drug treatments with the consequent development of side effects. Furthermore, skin diseases require a lot of attention, since they are associated with expensive treatments that are usually ineffective [1]. Studying the use of cells as a therapeutic agent instead of conventional drugs, for the control of these patients with dermatological problems, is therefore of special interest.

This review analyzes the most relevant stem cell types in skin regeneration and specific dermatological that may benefit from treatment with this new therapeutic approach.

2. The skin and the wound healing process

The skin is the main barrier that protects the body from the external environment, maintaining the homeostasis and with self-healing capacity. It is a complex organ, with different layers (epidermis, dermis, and hypodermis), that upon the loss of integrity, whether due to a disease or a lesion, needs to re-establish its function [1].

The wound healing process is a complex cascade of events that must occur in sequence and at the adequate time, in order to be successful. It has four overlapping phases: hemostasis, inflammation, proliferation, and remodeling. After a lesion, the first reaction of the body is to prevent blood loss, using the platelets to form a blood clot (hemostasis). Then, the inflammatory phase starts by recruiting inflammatory cells into the lesion site that will produce growth factors, cytokines and enzymes, increasing the temperature, redness, swelling, and local pain. If this phase extends in time, there will be a chronic inflammation that will harm the wound healing. The next phase is the proliferative, which consists in covering and filling the void space created by the lesion. For this, wound contraction occurs by local fibroblasts that differentiate into myofibroblasts. In addition, endothelial cells proliferate and migrate to form

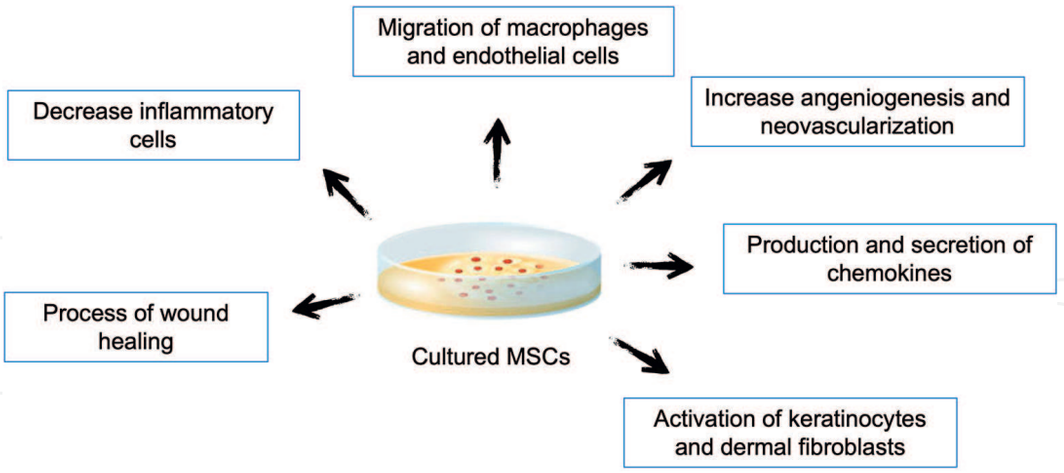

Figure 1.
Wound healing cascade and scar formation.

new blood vessels at the lesion site. It starts in the 4th day post-injury and can last for 2 weeks. Then, the fourth phase (remodeling) occurs and extracellular matrix deposits, promoting the re-epithelization and neovascularization, as the collagen fibers change from type III to type I, helping the tissue to remodel and regain its flexibility and tensile strength. This phase begins 2–3 weeks post-lesion and can last for several years [1, 8, 9].

The wound healing process can sometimes fail and, although the process is not fully understood, the prolonged chronic local inflammation is associated with an abnormal regeneration, as it supports the formation of scars, as demonstrated in **Figure 1**. There are also several factors that increase the risk of inappropriate wound healing, such as smoking, malnutrition, infections, age, metabolic diseases, medications, and even radiation [1, 8]. Tissue engineering, using stem cell-based therapies is being explored in various research fields obtaining good outcomes. Stem cells have gained a lot of attention because of some of their capacities, such as differentiation and the ability to aid tissue regeneration [10].

3. Types of stem cells

Depending on their potential, stem cells can be classified into three types: totipotent (cells have the ability to originate all types of cells from the three germ layers—endoderm, mesoderm, and ectoderm and extra-embryonic tissues); pluripotent (cells can originate cells from the three germ layers but not cells from extra-embryonic tissues), and multipotent (cells can originate cells from several types of tissues but only from one of the germ layers) [3]. Furthermore, stem cells can be collected from two major types of tissues, namely embryonic tissues and adult tissues. Their collection using biotechnology is a complex and costly process [11].

Embryonic stem cells (ESC) can be obtained from the inner cell mass of an early embryo. When removed, these cells can be cultured *in vitro* and have immortal characteristics. In addition, ESC may be induced to originate various cell/tissue types. For these reasons, ESCs are studied to better understand the mechanisms of organ formation and healing. Despite this, these cells can promote the formation of teratomas and can be rejected, when implanted into a patient, and there is some ethics controversy about the use of embryos in science [12].

Characteristics	Adult stem cell	Embryonic stem cell
	BM-MSCs, ADSCs, UC-MSCs	Inner cell mass of an early embryo
Teratomas formation	✗	✓
Rejected as foreign tissue	✗	✓
Differentiation ability	✓	✓
Self-renewal	✓	✓
Immortal cell lines	✗	✓

Table 1.
Summary of Adult Stem cells and Embryonic Stem cells and general characteristics.

Adult stem cells (ASC) can be found in almost every tissue, including adipose tissue, skin, bone marrow, muscle, among others. Some ASC, especially MSCs, can produce growth factors and can differentiate into many lineages. As opposed to ESC, ASCs do not lead to the formation of teratomas, unless there has been some damage prior to its implantation. These different characteristics between ESC and ASCs are compared in **Table 1**. Neonatal stem cells from the amnion, placenta, and umbilical cord are commonly considered as ASCs [12].

Among all these sources, bone marrow-derived MSCs (BM-MSCs) and adipose-derived MSCs (ADSCs) are the most studied and used in veterinary medicine due to the ease of obtaining, abundance of tissue of origin, and lack of moral restrictions [13].

4. Mesenchymal stem cells

MSCs were firstly characterized by Friedenstein's group as being phenotypically identical to fibroblasts and capable to adhere to plastic surfaces [1]. These cells are defined by the International Society for Cellular Therapy as cells that express specific surface markers (CD73, CD90, and CD105), do not exhibit the hematopoietic markers (CD45, CD34, CD14, CD19, CD11b, CD79a, and others), and have the ability to adhere to plastic surfaces when in culture and multipotential ability to differentiate in at least osteoblasts, chondrocytes, and adipocytes under specific *in vitro* conditions [14]. MSCs can be isolated from various sources such as adipose tissue, bone marrow, umbilical cord, dental pulp, olfactory mucosa, and muscle [15, 16].

In addition to their ability to differentiate into various types of tissues, MSCs can be used to produce secretome, which is composed by a wide variety of secreted

bioactive substances such as proteins, cytokines, growth factors, antioxidants, proteosomes, and exosomes that interact in an autocrine and paracrine way. MSCs secretome is an alternative therapeutic option and can help solving some limitations related to the use of living cells, such as tumorigenicity, immune compatibility, and infection transmission [10].

Among the numerous performance capabilities of MSCs, it is important to highlight their high capacity to differentiate into various cell types such as osteoblasts, chondrocytes, adipocytes, hepatocytes, myocardial cells, endothelial, neuronal, and epithelial cells, helping in the regeneration of damaged tissues. The potential to secrete cytokines and growth factors can promote angiogenesis and neo-vascularization, thereby increasing tissue blood flow, and the anti-apoptosis characteristics through the production of cellular factors that promote cellular survival prevent apoptosis or programmed cell death. In addition, MSCs can migrate to damaged areas of the body where they can act in tissue repair, which allows its local application directly *in situ* or *via* systemic administration. Their anti-inflammatory action and the inhibition of pro-inflammatory factors, as well as the immunomodulatory potential, make these cells good candidates to the treatment of dermatological disorders, as described in the characteristics in **Figure 2** [3, 14, 17, 18].

4.1 Immuno-modulating capacity of MSCs

MSCs act on different types of cells of the immune system by releasing more than 200 bioregulatory substances with antifibrotic, antiapoptotic, antimicrobial, chemoattraction, stem cell support, hematopoietic, angiogenesis, mitogenesis and neuroprotector properties [7]. In addition, MSCs have two fundamental effects on the immune system, which are an immune-enhancing and anti-inflammatory response [3]. These cells interact with T cells, B cells, natural killer (NK) cells, dendritic cells (DCs) macrophages, monocytes, and neutrophils, exerting immunoregulatory action on the innate and adaptive immune response [17].

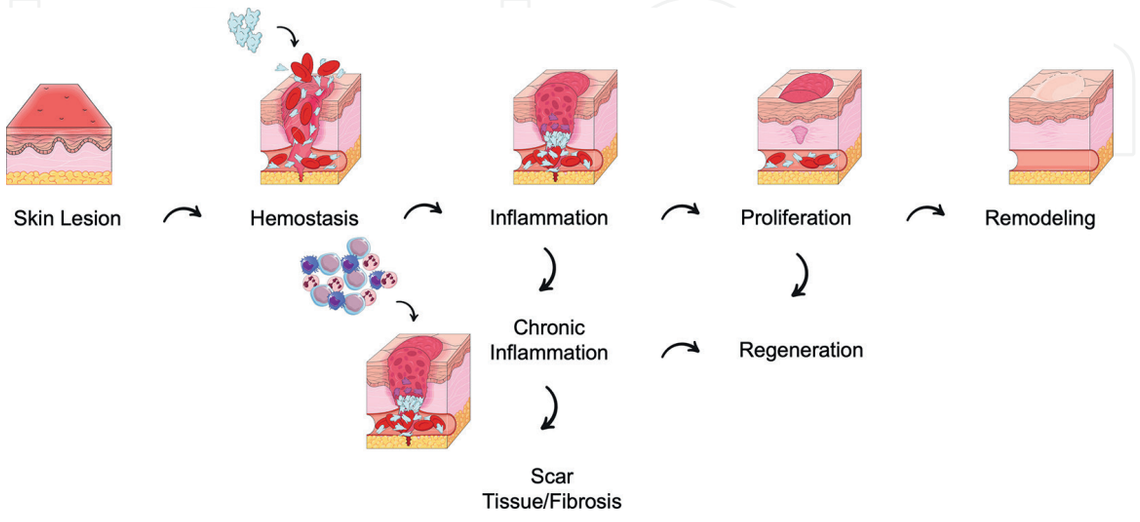

Figure 2.
MSCs role in the wound healing process.

The immunoregulatory potential of MSCs depends on several factors, such as their tissue of origin, MSC dose, administration time, MSC activation, and their contact with immune system cells.

4.2 Mechanism of action of MSCs under the innate immune response

The innate immune response is the body's first line of defense against any external action produced by pathogenic agents such as bacteria, fungi, and virus. It is a fast-acting and nonspecific response to those pathogens. This defense process causes tissue inflammation through the activation of immune system cells such as neutrophils, macrophages, monocytes, natural killer and dendritic cells, and the release of enzymes that form the complement system [19].

MSCs secrete prostaglandin E2 (PGE2), transforming growth factors (TGF-B), and indolamine2,3-dioxygenase that can modulate NK, inhibiting their proliferation, cytokine release, and cytotoxicity. This mechanism can also be exerted through cell-to-cell contact. In addition, MSCs also act on monocytes and macrophages. PGE2, TGF-B, hepatic growth factor, interleukin 6 released by MSCs, reprogram macrophages with a pro-inflammatory M1 phenotype to an anti-inflammatory M2 phenotype with increased production of interleukin-10, and decreased production of tumor necrosis factor and gamma interferon [20–22].

The release of these same soluble factors (PGE2, TGF-B, and interleukin 6) acts on monocytes by inhibiting their differentiation into dendritic cells. Dendritic cells are antigen-presenting cells, when their maturation is inhibited, the correct expression of presenting and co-stimulatory molecules does not occur, which results in a lack of response on the part of T cells [3, 23]. Furthermore, MSCs have the capacity to inhibit the infiltration of monocytes, macrophages, and neutrophils into sites of inflammation, dependent on the tumor necrosis factor stimulated gene 6 protein (TSG6). Similarly, MSCs can also enhance the infiltration of the cells into tumors in a chemokine-dependent manner. In this case, MSCs can promote tumor progression, metastasis, and treatment resistance. For instance, the stimulation of chemokine production may stimulate the capacity of MSCs to attract macrophages, monocytes, and neutrophils. Conversely, an inflammation stage might activate the expression of indoleamine-2,3-dioxygenase (IDO) produced by MSCs that can cause immunosuppressive consequences on myeloid cell migration. For these reasons, it is hard to predict whether the immunomodulatory response of MSCs is expected to be negative or positive, because of MSCs complex innate immune cell interactions [23].

4.3 Mechanism of action of MSCs under the adaptive immune response

The adaptive immune response develops a defense mechanism specific for each pathogen. Therefore, a memory effect is created for each antigen after the first contact, in order to develop a faster and more effective response the next time the organism is in contact with the same antigen [3].

The immune system acts through two pathways, the cellular immune response composed of T lymphocytes that directly attack the pathogens that invade the organism and the humoral immunity response composed of antibodies against pathogenic antigens produced by B lymphocytes [22].

One of the major mechanisms of action of MSCs is their ability to regulate T cells through cell-to-cell interaction or secretion of inflammatory components. In this

environment, MSCs can change from T-helper 1 (Th1) phenotype (proinflammatory) into a T-helper 2 (Th2) phenotype (anti-inflammatory) [3]. There is some contradiction about the effects of MSCs on B cells, although there is clear evidence that MSCs have close interaction with these cells. Thus, MSCs are capable of inhibiting B cell proliferation through cell-to-cell contact and with the arrest in the cell cycle. MSCs can regulate immune responses, but their immunomodulatory capacity is not yet fully understood.

This anti-inflammatory and immunomodulatory capacity of MSCs is very promising for the treatment and recovery of skin tissue [14].

5. Application of cell-based therapies in veterinary dermatology

Slow wound healing or persistent wounds are a challenge for clinicians, in both veterinary and human medicines. These wounds can result in inadequate tissue reorganization, culminating in a long period of incapacity and unsatisfactory outcomes [24]. These conditions can be related to various pathological conditions, such as autoimmune diseases, diabetes, and venous stasis, for which no definitive therapies are currently available [25]. Due to this situation, the use of MSCs in veterinary medicine has been increasing in the recent years in different fields. Regarding their application in dermatology, these cells can be used for skin tissue regeneration and for the control of dermatological pathologies, in which the immune system intervenes [14]. Due to their capacity for regeneration, differentiation, revascularization, as well as, their anti-inflammatory and immunomodulatory properties, MSCs have been used as promoters of regeneration on tissues that suffered some damage [1]. However, there are still some questions regarding the use of MSCs, such as their immunomodulation mechanism that is not fully understood. In addition, there are different routes of administration that can result in different risks for the patient. For instance, systemic administration can lead to the entrapment of MSCs in the lung or microvasculature that can cause side effects, such as pulmonary emboli. In addition, almost 90% of the cells are lost, once administered, because of hypoxia, inflammation, physical stress, or immunogenic rejection. For this reason, to reach a therapeutic efficacy, a huge number of cells may be needed, increasing the risk of teratoma formation. Therefore, new studies are needed to achieve more cost-effective treatments to overcome these obstacles [3]. Despite these, this review demonstrates the positive effects of using MSCs therapies and the need to standardize protocols.

There are several skin diseases that can produce chronic and recurring wounds, such as canine atopic dermatitis (CAD), pemphigus foliaceus (PF), and perianal fistula. The conventional treatment for the skin diseases consists in glucocorticoids, cyclosporine, and oclacitinib, which are used due to their immunomodulatory effect. However, their repeated use has several side effects, such as polydipsia, polyuria, polyphagia, vomiting, and diarrhea. It can also require increasing doses of medication over time due to drug habituation [3, 26].

5.1 Application of MSCs in tissue repair and chronic non-healing wounds

A wound is a disruption of the functional integrity and anatomic structure of the skin, so wound healing is a highly ordered process. The skin tissue repair steps need to occur in consecutive order and timing, to be successful; otherwise, the healing process will fail and cause complications, such as chronic non-healing wounds [1].

There are various types of non-healing wounds, such as bed sores, diabetic foot, and trophic ulcers of many etiologies. For instance, ulcer treatment requires necrotic tissue debridement, wound cleansing, amelioration of damaging factors (infection), improvement of arterial blood circulation, and medical management to aid with the comorbidities. Nonetheless, chronic wounds take a long time to heal and often recur after healing with extensive and intensive treatment. Thus, a great possibility in the treatment of chronic wounds is associated with cell-based therapies [27].

The use of MSCs in wound healing is due to their ability to remove necrotic and dead cells, improve vascularization and re-epithelization, and diminish scar formation and wound contraction. Transplanted MSCs release several growth factors that help coordinate different repairing activities. Fibroblasts, endothelial cells, and local stem cells are triggered to aid in tissue repair, by increasing angiogenesis, restraining leukocyte transmigration, and stimulating the proliferation, migration, and differentiation of keratinocytes and fibroblasts. MSCs also release immunosuppressive factors, helping to suppress the proliferation of immune cells, reducing inflammation, and, consequently, reducing scar formation [1].

Kuperman *et al.* demonstrated that the local application of mouse oral mucosa stem cells (mOMSCs) increased the wound healing rate and re-epithelialization and led to a larger area of granulation tissue in diabetic mice compared to the control group [28].

Gorecka *et al.* applied human-induced pluripotent stem cell-derived smooth muscle cells (hiPSC-SMCs) embedded into 3D collagen scaffolds in diabetic mice, promoting angiogenesis and accelerating diabetic wound healing [29].

A study in rabbits showed that wounds treated with a combination of plasma rich in growth factors and adipose-derived mesenchymal stem cells (PRGF+ADSCs) have higher wound healing and epithelization rates, less inflammation and scar tissue, greater collagen deposits, and better angiogenesis compared to the control group. Furthermore, the group treated with the combination PRGF+ADSCs showed a faster recovery of the damaged tissue [30].

Several studies in rodent models have demonstrated that MSCs applied subcutaneously, topically, or intravenously can improve wound healing [12, 31–33].

In a study focusing on skin wounds in dolphins, the animals were treated with autologous ADSCs in a blinded clinical study and the group treated with ADSCs showed improved wound healing [12].

The use of secretome is an alternative treatment when dealing with chronic skin wounds. Sue *et al.* used a rat skin excisional wound healing model to demonstrate that the subcutaneous injection of ADSCs secretome around the wound could accelerate cutaneous wound healing [34].

Park *et al.* investigated if ADSCs secretome could accelerate wound healing using nude mice. In this study, a full-thickness excisional skin wound was created bilaterally on the dorsal surface of the animal. Then, the secretome was used topically in the wounds and covered with a transparent dressing. The evaluation of the lesions demonstrated that the treatment using secretome was able to stimulate angiogenesis, skin thickening, and the recruitment of immune cells, therefore enhancing the wound healing process [35].

5.2 Application of MSCs in immune-mediated diseases

CAD is a complicated disease that results from environmental factors (allergens) and a genetic predisposition (filaggrin mutation) that alter the immune response and

culminate in a skin barrier dysfunction [36]. It is a common multifactorial inflammatory and pruritic skin disease in dogs, associated with the production of IgE antibodies [37]. Its prevalence is around 10–15%, and the management of these patients is a real challenge for tutors and veterinarians [38]. During the acute phase of CAD, there is an activation of Th2 cells caused by the immune dysregulation, which culminates in the production of numerous pro-inflammatory cytokines. Over time, the chronicity of the pathology is maintained by a broader roster of T helper variations. Pharmacological treatments consist in corticosteroids (less specific) and cyclosporine A (more specific), and some new approaches used to achieve the most target agents, such as lokivetmab and oclacitinib. Despite the newest therapies, approximately 25–40% of dogs with CAD endure clinical signs and do not reach a full resolution of the pathology [39]. For that reason, the use of MSCs would be a good alternative therapy, since their use in an inflammatory environment, can alter the cytokine profile of T cells and dendritic cells, which can lead to an anti-inflammatory environment [12].

In 2018, a study gathered 26 animals diagnosed with CAD refractory to conventional treatments to which an intravenous dose of 1.5×10^6 ADSCs/kg bodyweight was administered. Pruritus was evaluated using the Canine Atopic Dermatitis Extent and Severity Index, version 4 (CADESI-4), and a decrease in pruritus within a time span of 1 week to 1 month was observed, with the animals being controlled for a period of 6 months. Owners reported improvement of the animals with a satisfactory global assessment of the treatment without the occurrence of adverse events [40].

In 2019, a group of 12 canine patients diagnosed with CAD were intramuscularly inoculated with 0.5×10^6 of cryopreserved ADSCs. Injections were repeated weekly for 6 weeks. During this period, the effectiveness of the treatment was evaluated by the pruritus index and by the CAD Lesion Index (CADLI) test, and a notable reduction in both was observed. The animals were monitored at all times, and no systemic side effects or changes at the injection site were observed [41].

In 2020, a group of 16 animals diagnosed with CAD was evaluated with CADESI-4 and divided into three groups, namely mild, moderate, and severe according to the severity of their injuries. For 82 days, $2x10^6$ MSCs were inoculated intravenously every 21 days to all animals. At the end of the 82 days, skin biopsy histopathology analysis was performed, observing a significant reduction in epidermal thickness in the moderate and severe groups. The results demonstrate that MSCs attenuated the clinical signs of CAD resulting in a safe therapy and causing no adverse effects [42].

In 2021, a double-blind study divided patients with CAD into three groups, a control group that received PBS solution, one group that received low-dose ADSCs (5×10^5 cells/kg), and the third group received a higher dose (5×10^6 cells/kg) of MSCs. Three subcutaneous treatments were performed at 4-week intervals. Pruritus was assessed by tutors using the pruritus visual analog scales (PVAS) and by veterinarians using CADESI-4. Both observed a decrease in pruritus during the 30 days following injections in the group receiving higher dose of MSCs. The animals were monitored throughout the study and did not manifest adverse side effects [43].

Recently, in 2022, a study evaluated the immunomodulatory effect of cADSCs and extracellular vesicles derived from cADSCs (cADSC-EVs) demonstrating that these cells have a beneficial effect in atopic animals. The cASCs and cADSC-EVs affect the expression levels of epidermal differentiation proteins, such as keratin1, filaggrin, loricrin, and involucrin promoting the recovery of the deficient skin barrier in these atopic animals. With the recovery of the skin barrier, it was possible to reduce the loss of water and prevent the entry of allergens *via* transepidermal route. cADSCs and cASC-EVs regulate the immune and inflammatory response

by regulating mast cell infiltration, decreasing serum levels of IgE, inflammatory cytokines and epidermal chemokines (IL-4, IL-13, and IL-31) that intervene in Th2. By decreasing IL-31, a decrease in pruritus was also observed by inhibiting the activation of JAK-STATs signaling [38].

The use of UC-MSCs secretome has been associated with the improvement of atopic dermatitis, due to the secretion of epidermal growth factor, which regulates the inflammatory response of mast cells, Th2 cells, and keratinocytes [44].

5.3 Application of MSCs in autoimmune skin diseases

Autoimmune skin diseases occur when the body itself causes self-destruction of its cells and tissues [45].

Pemphigus describes a group of cutaneous autoimmune diseases. Pemphigus vulgaris consists in the appearance of mucocutaneous blisters that are characterized by the presence of autoantibodies against desmogleins (1 and 3). It can be life threatening, and the treatment with systemic corticosteroids has improved the mortality rates [46].

PF is a common autoimmune skin disease characterized by acantholysis. This pathology is associated with the production of autoantibodies that target protein in the desmosomes of keratinocytes [47]. The desmoglein-1 and the desmocollin-1, which are epidermal adhesion proteins, are the main antigens implicated in PF [48]. It is characterized by the production of self-antibodies by B cells that attack desmoglein-1, causing cell apoptosis and disruption of the skin layers, resulting in vesicles, intra-epidermal pustules, and crust lesions [49]. The cause is usually unknown, but some situations are a sequel to a chronic inflammatory skin disease or probably drug-induced [47]. The treatment and control of PF is difficult and often requires lifelong therapy with immunosuppressive drugs (corticosteroids or cyclosporine), having severe side effects such as polyuria/polydipsia, diarrhea, weigh gain, and predisposition to recurrent infection [50]. Furthermore, it has been described that only 53% of treated cases of PF survive more than 1 year after the treatment initiation [47]. Due to their immunomodulatory, anti-inflammatory, and antiapoptotic abilities, MSCs are a promising therapeutic option, not showing the side effects of the conventional treatment.

In 2015, a 10-year-old neutered Shih-Tzu dog diagnosed with PF refractory to corticosteroid treatment was treated with cytotoxic T-lymphocyte antigen 4 (CTLA4)-overexpressing ADSCs. CTLA4-ADSCs and/or naive ADSCs were administered 21 times over a 20-month period (every 2 to 8 weeks) with a positive result. The prednisolone dose was progressively lowered with no relapse of the lesions. At the end of treatment, the lesions had improved considerably, and the disease was under control with a low dose of corticosteroids for 12 months [47].

Canine anal furunculosis is a chronic inflammatory disease that leads to perianal fistulas and that shares a large part of etiology and clinical manifestation with Crohn's disease in humans [51].

In 2015, six dogs with perianal fistulas were treated with human embryonic stem cell-derived mesenchymal stem cells (hESC-MSCs). One month after the injection, reduced serum levels of IL-2 and IL-6 and two inflammatory cytokines associated with Crohn's disease were observed, and after 3 months all animals were controlled and remained free of fistulas for 6 months [51].

Psoriasis is another chronic autoimmune disease, characterized by silvery white scaly patches projecting from the inflamed skin, mainly present in humans, but that can also appear in dogs and monkeys [52]. In patients with psoriasis, the histology of damaged skin demonstrates that epidermal keratinocytes are hyperproliferative and

a huge number of immune cells infiltrate into the skin layers (dermis and epidermis). The first treatment used for this pathology was crude coal tar or a combination of the last with ultraviolet irradiation, both with the purpose to restrain keratinocyte proliferation and therefore restoring normal epidermopoiesis. With time, retinoids were also proved to be an efficient treatment for psoriasis since they were able to inhibit the keratinocytes proliferation. For that reason, in the beginning keratinocytes were considered as the main inducer of psoriasis. Later, cyclosporine was discovered to be efficient in psoriasis treatment by inhibiting cytokines produced by T cells [53]. Therefore, keratinocytes can trigger psoriasis and actively participate in a complex environment coordinated by cytokines [54]. The treatments depend on the severity of the disease, from topical agents and phototherapy to the usual immunosuppressant drugs [55]. However, these therapies are expensive and are related to adverse reactions, and therefore, there is a need to discover a more effective and safer treatment. For that reason, there are some expectations in the use of MSCs for psoriasis.

A study testing a treatment with umbilical cord-derived MSCs (UC-MSCs) resulted in comparison with the conventional treatments (betamethasone cream) on imiquimod-induced psoriasis-like skin lesion in adult male albino rat model. MSCs showed efficacy in reducing the severity of the disease, demonstrating that the cells had anti-inflammatory and immunomodulatory effects by inhibiting the clinical manifestation [55].

The use of the MSCs secretome in psoriasis is also able to inhibit the maturation and activation of DC and IL17, decreasing psoriasis score in a rat model [56].

Systemic lupus erythematosus (SLE) is a multisystemic autoimmune disease, with clinical manifestations on every organ. The production of antigen-antibodies complexes that settle in the basement membrane zone of the skin can make this disease manifest as cutaneous lupus erythematosus, with the appearance of rashes. It is a multifactorial disease that can be caused by genetic factors, B cell hyperactivity, T cell defects, virally induced antigen-antibody complex formation, and even hormonal alterations [57]. The cutaneous inflammation is due to Th1 cells, neutrophils, B cells, and even complex cascades of native skin cell types (keratinocytes and endothelial cells) [58]. The conventional treatment options consist in the use of corticosteroids and cyclophosphamide.

The use of allogenic MSCs administrated intravenously has improved multiorgan dysfunction in both MRL/lpr mice and NZB/W1 F1 mice, used as SLE animal models [59].

The use of human AD-MSCs also improved the survival rate and histological, serological, and immunological function of NZB × NZW mice, models for SLE, without adverse effects [57].

5.4 Application of MSCs in alopecia

Alopecia is a frequent dermatologic disorder, affecting both veterinary species and humans. The pathophysiology of this group of disorders is still unclear, but can be associated with trauma, stress, autoimmune disorders, hormones, and even genetics [60]. Alopecia can be caused by a several disorders, such as infections, allergenic reasons, nutritional deficits (mineral deficiency), parasites, bacteria or hot spots, and fungi. A complete physical examination and history are needed to an accurate diagnosis for the reason of hair loss [61]. Hair loss is usually accompanied by a section of thinned skin at the site with hair loss. Additionally, these clinical symptoms occur because of the functional loss of follicular stem cell activity. Nowadays, there are various treatments for alopecia, such as oral medications, that only improve the situation

temporary and often with reduced effectiveness. For this reason, alternative strategies are fundamental for alopecia [62]. The use of BM-MSCs and UC-MSCs *in vitro* and in an athymic nude mouse model has been able to create dermal papilla-like structures, as well as hair follicles [12]. In a study, the use of UC-MSCs increased the regeneration of new follicles and improved onset of anagen phase [60]. To determine if UC-MSCs promote the hair cycle, these cells were injected intra-dermally at multiple sites in the dorsal skin field of depilated mouse. The control was the use of daily topical treatments with 3% minoxidil, and the hair cycle stage was determined with the measurement of skin hair regrowth. The results showed that the UC-MSCs emphasized hair follicle morphogenesis, but only without the ablation of the neonatal dermal cell, indicating that UC-MSCs facilitate hair growth and regeneration *via* a paracrine mechanism [62]. Several studies using ADSCs secretome had positive results when used to treat alopecia, as they were able to restore hair loss, due to the activation of hair regeneration pathways [63, 64].

5.5 Application of MSCs in scar tissue

Scar tissue is produced due to cutaneous wound healing, in which there is excessive deposition of extracellular matrix, allowing the skin to restore its integrity after a lesion. It occurs due to specific mechanisms that are modulated by local pro-inflammatory mediators, in the inflammatory phase of wound healing [65]. It has a different appearance, and the tissue does not function as normal skin, lacking some structures, such as hair follicles, sensory nerve receptors, and even sebaceous glands. Its tensile strength is also diminished in about 20%, which leads to re-injures, as the scar is considered a weak point [65].

The current treatments include sterile dressing and topical antibiotics to diminish infection risk and to advance into the proliferation and remodeling phases of the wound healing process, then allowing the regeneration of the tissue. It may also be necessary to debride the wound and irrigate it, to remove necrotic tissue [65].

The use of ADSCs has been reported to have the capacity to remodel scar tissue or block its formation, when used with a fat graft, in human plastic surgery [12].

A study has shown that the use of silk fibroin scaffolds containing human Wharton's jelly MSCs (Wj-MSCs-SF) in a murine model reduced the formation of fibrotic scar tissue, improving reepithelization and vascularized granulation tissue [66].

Gentile *et al* have demonstrated that the use of ADSCs contained in fat grafts has positive effects in scar signs and symptoms [67].

The use of ADSCs secretome in full skin defects demonstrated that it is capable of reducing scar formation, as well as accelerate wound closure and improve angiogenesis [68].

5.6 Application of MSCs in burns

Burns are very common lesions both in veterinary and human medicine and occur due to thermal, electrical, chemical, or radiation exposure. They are associated with the loss of normal tissue and cells, which complicates the wound healing process. Their severity is characterized by the depth and size of tissue damage, as first-, second-, third-, or fourth-degree burns. A first-degree burn is red or pink and sensitive, and can swell slightly. A second-degree burn is painful and often associated with the presence of blisters that reach the reticular dermal layer and have a high risk of chronic inflammation and keloid/hypertrophic scar formation. A third-degree burn

presents necrosis of the skin and deep tissues, and is compact and immobile. Fourth-degree burns presents as dry, very compact, and wrinkled. There are alterations in blood composition associated with a burn that occupies 10% or more of the body, which leads to metabolic complications [69, 70].

Current treatments consist in antibiotics, fluids, and detoxification. First- and second-degree burns are treated with antibiotics and anti-inflammatory drugs. Third- and fourth-degree burns require regular dressing change and sometimes surgery [70]. In several studies, the use of local injections of UC-MSCs and ADSCs in rodent models has diminished burn wound progression, as well as burn-induced inflammation. They have also accelerated wound healing in burned lesions, increased re-epithelization, vascularization, and granulation tissue formation [71–73].

The use of a hydrogel containing unsaturated arginine-based poly(ester amide) and chitosan associated with BM-MSCs also demonstrated an increased re-epithelization, granulation tissue formation, and wound healing rate [73, 74]. Also, the use of systemically delivered BM-MSCs in rodent models has increased wound healing [73].

Hackers *et al.* demonstrated the effects of secretome from peripheral blood mononuclear cells on pig models of skin burns. The results showed a decrease in the amount of mast cells in the wound area, demonstrating a lower inflammation of the burn area when using MSCs secretome [75].

In the study by Kudinov et al. using secretome from UC-MSCs combined with a chitosan hydrogel in burned rats, the results showed that this combination cleared the wound of bacteria, promoted re-epithelization and the formation of vascularized granulation tissue, and decreased inflammation [76].

6. Conclusion

Dermatological pathologies are increasingly common and often have a chronic nature, resulting in a real challenge to owners and veterinaries. This leads to animals having to receive long-term or lifelong medications with its consequent systemic side effects. Veterinary regenerative medicine is an area field of research that is becoming more explored and active. Therefore, in the recent years, there have been significant advances in developing effective and safe stem cell therapies. Those studies reveal that MSCs have anti-inflammatory, anti-apoptotic, anti-fibrotic, and immunomodulatory potential, which ensures success in the treatment of degenerative, immunological, and inflammatory diseases in animals. In this review, some relevant cited studies in the field of veterinary dermatology demonstrated better results in the treatment of chronic wounds, especially with the use of UC-MSCs and ADSCs. These studies also showed accelerated wound healing in different clinical conditions. Although more studies are still necessary in this regard, the use of MSCs seems to be a very promising and safe therapeutic option.

Acknowledgements

All authors had made substantial contributions to the work, with well-established division of tasks. All authors reviewed the final work and approved its submission. All authors agreed to be personally accountable for the author's own contributions and for ensuring that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately

investigated, resolved, and documented in the literature. All authors have read and agreed to the published version of the manuscript.

This research was funded by Projects PEst-OE/AGR/UI0211/2011 and LA/P/0059/2020 funded by the Portuguese Foundation for Science and Technology (FCT), and COMPETE 2020, from ANI–Projetos ID&T Empresas em Copromoção, by the project "Print-on-Organs–Engineering bioinks and processes for direct printing on organs" with the reference POCI-01-0247-FEDER-033877, by the project "Bone2Move-Development of 'in vivo' experimental techniques and modelling methodologies for the evaluation of 4D scaffolds for bone defect in sheep model: an integrative research approach" with the reference POCI-01-0145-FEDER-031146 and by the PhD scholarships Mariana Vieira Branquinho (SFRH/BD/146172/2019), Ana Catarina Sousa (SFRH/BD/146689/2019), and Bruna Lopes (2021.05265.BD). The author Rui D. Alvites acknowledges Centro de Estudos de Ciência Animal (CECA), Instituto de Ciências, Tecnologias e Agroambiente (ICETA), Porto University (UP), and Fundação para a Ciência e Tecnologia (FCT) for the funding and availability of all technical, structural, and human resources necessary for the development of this work. The author Patrícia Sousa acknowledges Instituto Politécnico de Leiria—Center for Rapid and Sustainable Product Development (CDRSP), University of Porto (UP), Centro de Estudos de Ciência Animal (CECA), Instituto de Ciências, Tecnologias e Agroambiente (ICETA) for the funding (UIDB/04044/2020) and availability of all resources needed for this work. The work was supported through the projects UIDB/04044/2020 and UIDB/00211/2020 funded by FCT/MCTES through national funds.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this chapter.

Abbreviations

ADSCs	Adipose-derived MSCs
ASC	Adult stem cells
BM-MSCs	Bone marrow-derived MSCs
CADLI	CAD lesion index
CAD	Canine atopic dermatitis
CADESI-4	Canine atopic dermatitis extent and severity lesion, version 4
CTLA4	Cytotoxic T-lymphocyte antigen 4
DCs	Dendritic cells
ESC	Embryonic stem cells
cASCs – EVs	Extracellular vesicles derived from cASCs
hESC-MSCs	Human embryonic stem cell-derived mesenchymal stem cells
hiPSC-SMCs	Human-induced pluripotent stem cell-derived smooth muscle cells
IDO	Indoleamine-2,3-dioxygenase
MCSs	Mesenchymal stem cells
mOMSCs	Mouse oral mucosa stem cells
NK	Natural killer
PF	Pemphigus foliaceus
PRGF	Plasma rich in growth factors

PGE2	Prostaglandin E2
PVAS	Pruritus visual analog scales
SLE	Systemic Lupus Erythematosus
Th1	T-helper 1
Th2	T-helper 2
THF-B	Transforming growth factors
TSG6	Tumor necrosis factor stimulated gene 6 protein
UC-MSCs	Umbilical cord-derived MSCs
Wj-MSCs-SF	Wharton's jelly MSCs

Author details

Carolina Mesquita[1,2,3†], Bruna Lopes[1,2,3†], Patrícia Sousa[1,2,3†], Mariana Branquinho[1,2,3], Ana Catarina Sousa[1,2,3], Ana Lúcia Luís[1,2,3], Rui Alvites[1,2,3,4] and Ana Colette Maurício[1,2,3*]

1 Departamento de Clínicas Veterinárias, Instituto de Ciências Biomédicas de Abel Salazar (ICBAS), Universidade do Porto (UP), Porto, Portugal

2 Centro de Estudos de Ciência Animal (CECA), Instituto de Ciências, Tecnologias e Agroambiente da Universidade do Porto (ICETA), Porto, Portugal

3 Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Lisboa, Portugal

4 Instituto Universitário de Ciências das Saúde – CESPU (IUCS-CESPU), Gandra, Portugal

*Address all correspondence to: acmauricio@icbas.up.pt

† The authors should be considered as first authors.

References

[1] Lopes B et al. The application of mesenchymal stem cells on wound repair and regeneration. Applied Sciences. 2021;**11**(7):3000

[2] Quinones-Vico MI et al. The role of exosomes derived from mesenchymal stromal cells in dermatology. Frontiers in Cell and Development Biology. 2021;**9**:647012

[3] Dias IE et al. Mesenchymal stem cells therapy in companion animals: Useful for immune-mediated diseases? BMC Veterinary Research. 2019;**15**(1):358

[4] Lopes B et al. Peripheral nerve injury treatments and advances: One health perspective. International Journal of Molecular Sciences. 2022;**23**(2):10-14

[5] Han Y et al. Mesenchymal stem cells for regenerative medicine. Cell. 2019;**8**(8):1-3

[6] Xing SJ et al. Isolation, characterization, and therapeutic application of extracellular vesicles from cultured human mesenchymal stem cells. Journal of Visualized Experiments. 2022;(187):1-2

[7] Ayala-Cuellar AP et al. Roles of mesenchymal stem cells in tissue regeneration and immunomodulation. Biomolecules and Therapeutics (Seoul). 2019;**27**(1):25-33

[8] Guillamat-Prats R. The role of MSC in wound healing, scarring and regeneration. Cell. 2021;**10**(7):1729

[9] Huang Y-Z et al. Mesenchymal stem cells for chronic wound healing: Current status of preclinical and clinical studies. Tissue Engineering Part B: Reviews. 2020;**26**(6):555-570

[10] Izzah N et al. Cell secretomes for wound healing and tissue regeneration: Next generation acellular based tissue engineered products. Journal of Tissue Engineering. 2022;**13**:1-21

[11] Rashid U et al. Characterization and differentiation potential of mesenchymal stem cells isolated from multiple canine adipose tissue sources. BMC Veterinary Research. 2021;**17**(1):388

[12] Harman RJ. Stem cell therapy in veterinary dermatology. Veterinary Dermatology. 2013;**24**(1):90-e24

[13] Voga M et al. Stem cells in veterinary medicine—Current state and treatment options. Frontiers in veterinary. Science. 2020;**7**:7

[14] Villatoro AJ et al. Allogeneic adipose-derived mesenchymal stem cell therapy in dogs with refractory atopic dermatitis: Clinical efficacy and safety. The Veterinary Record. 2018;**183**(21):654

[15] Alvites RD et al. Rat olfactory mucosa mesenchymal stem/stromal cells (OM-MSCs): A characterization study. International Journal of Cell Biology. 2020;**2020**:1-21

[16] Mao AS, Mooney DJ. Regenerative medicine: Current therapies and future directions. Proceedings of the National Academy of Sciences of the United States of America. 2015;**112**(47):14452-14459

[17] Peroni JF, Borjesson DL. Anti-inflammatory and immunomodulatory activities of stem cells. The Veterinary Clinics of North America. Equine Practice. 2011;**27**(2):351-362

[18] Strong AL, Neumeister MW, Levi B. Stem cells and tissue engineering:

Regeneration of the skin and its contents. Clinics in Plastic Surgery. 2017;**44**(3):635-650

[19] Glenn JD, Whartenby KA. Mesenchymal stem cells: Emerging mechanisms of immunomodulation and therapy. World Journal of Stem Cells. 2014;**6**(5):526-539

[20] Kim N, Cho SG. New strategies for overcoming limitations of mesenchymal stem cell-based immune modulation. International Journal of Stem Cells. 2015;**8**(1):54-68

[21] Li H, Dai H, Li J. Immunomodulatory properties of mesenchymal stromal/stem cells: The link with metabolism. Journal of Advanced Research. 2022;**45**:15-29

[22] Carrade DD, Borjesson DL. Immunomodulation by mesenchymal stem cells in veterinary species. Comparative Medicine. 2013;**63**(3): 207-217

[23] Shi Y et al. Immunoregulatory mechanisms of mesenchymal stem and stromal cells in inflammatory diseases. Nature Reviews. Nephrology. 2018;**14**(8):493-507

[24] Lanci A et al. Heterologous Wharton's jelly derived mesenchymal stem cells application on a large chronic skin wound in a 6-month-old filly. Frontiers in Veterinary Science. 2019;**6**:6

[25] Marfia G et al. Mesenchymal stem cells: Potential for therapy and treatment of chronic non-healing skin wounds. Organogenesis. 2015;**11**(4):183-206

[26] Arnhold S et al. Biological macromolecules and mesenchymal stem cells: Basic research for regenerative therapies in veterinary medicine. International Journal of Biological Macromolecules. 2019;**123**:889-899

[27] Suzdaltseva Y et al. Locally delivered umbilical cord mesenchymal stromal cells reduce chronic inflammation in long-term nonhealing wounds: A randomized study. Stem Cells International. 2020;**2020**:5308609

[28] Kuperman S et al. Examination of the therapeutic potential of mouse Oral mucosa stem cells in a wound-healing diabetic mice model. International Journal of Environmental Research and Public Health. 2020;**17**(13):8-9

[29] Gorecka J et al. Induced pluripotent stem cell-derived smooth muscle cells increase angiogenesis and accelerate diabetic wound healing. Regenerative Medicine. 2020;**15**(2):1277-1293

[30] Chicharro D et al. Combined plasma rich in growth factors and adipose-derived mesenchymal stem cells promotes the cutaneous wound healing in rabbits. BMC Veterinary Research. 2018;**14**(1):288

[31] Sasaki M et al. Mesenchymal stem cells are recruited into wounded skin and contribute to wound repair by transdifferentiation into multiple skin cell type. Journal of Immunology. 2008;**180**(4):2581-2587

[32] Javazon EH et al. Enhanced epithelial gap closure and increased angiogenesis in wounds of diabetic mice treated with adult murine bone marrow stromal progenitor cells. Wound Repair and Regeneration. 2007;**15**(3):350-359

[33] Chen L et al. Analysis of Allogenicity of mesenchymal stem cells in engraftment and wound healing in mice. PLoS One. 2009;**4**(9):e7119

[34] Su N et al. Fibrous scaffolds potentiate the paracrine function of mesenchymal stem cells: A new

dimension in cell-material interaction. Biomaterials. 2017;**141**:74-85

[35] Park SR et al. Stem cell Secretome and its effect on cellular mechanisms relevant to wound healing. Molecular Therapy. 2018;**26**(2):606-617

[36] Marsella R. Advances in our understanding of canine atopic dermatitis. Veterinary Dermatology. 2021;**32**(6):547-e151

[37] Hensel P et al. Canine atopic dermatitis: Detailed guidelines for diagnosis and allergen identification. BMC Veterinary Research. 2015;**11**(1):196

[38] Kim SY et al. Mesenchymal stem cells and extracellular vesicles derived from canine adipose tissue ameliorates inflammation, skin barrier function and pruritus by reducing JAK/STAT Signaling in atopic dermatitis. International Journal of Molecular Sciences. 2022;**23**(9):2-3

[39] Black L et al. The effect of uterine-derived mesenchymal stromal cells for the treatment of canine atopic dermatitis: A pilot study. Frontiers in Vetenary Science. 2022;**9**:1011174

[40] Klinger C. Mesenchymal stem cells: A potential therapy for canine atopic dermatitis? The Veterinary Record. 2018;**183**(21):651-653

[41] Enciso N et al. Multidose intramuscular allogeneic adipose stem cells decrease the severity of canine atopic dermatitis: A pilot study. Vetenary World. 2019;**12**(11):1747-1754

[42] de Oliveira Ramos F et al. Canine atopic dermatitis attenuated by mesenchymal stem cells. Journal of Advanced Veterinary and Animal Research. 2020;**7**(3):554-565

[43] Kaur G et al. A double-blinded placebo-controlled evaluation of adipose-derived mesenchymal stem cells in treatment of canine atopic dermatitis. Veterinary Research Communications. 2022;**46**(1):251-260

[44] Jung N et al. Immunomodulatory effect of epidermal growth factor secreted by human umbilical cord blood-derived mesenchymal stem cells on atopic dermatitis. International Journal of Stem Cells. 2022;**15**(3):311-323

[45] Chase K et al. Understanding the genetics of autoimmune disease: Two loci that regulate late onset Addison's disease in Portuguese water dogs. International Journal of Immunogenetics. 2006;**33**(3):179-184

[46] Agarwal A et al. Comparison of rituximab and conventional adjuvant therapy for pemphigus vulgaris: A retrospective analysis. PLoS One. 2018;**13**(9):e0198074

[47] Han SM et al. CTLA4 overexpressing adipose tissue-derived mesenchymal stem cell therapy in a dog with steroid-refractory pemphigus foliaceus. BMC Veterinary Research. 2015;**11**:49

[48] Chong E, Austel M, Banovic F. A retrospective evaluation of the steroid-sparing effect of Oral modified Ciclosporin for treatment of canine pemphigus Foliaceus. Veterinary Sciences. 2022;**9**(4):153

[49] Olivry T. A review of autoimmune skin diseases in domestic animals: I - superficial pemphigus. Veterinary Dermatology. 2006;**17**(5):291-305

[50] Putra A, Austel M, Banovic F. A retrospective evaluation of the steroid sparing effects of oral mycophenolate mofetil (MMF) as an adjunct immunosuppressant for the treatment of canine pemphigus foliaceus. Veterinary Dermatology. 2022;**33**(1):77-e24

[51] Ferrer L et al. Treatment of perianal fistulas with human embryonic stem cell-derived mesenchymal stem cells: A canine model of human fistulizing Crohn's disease. Regenerative Medicine. 2016;**11**(1):33-43

[52] Sandra, R.M., et al., Psoriatic animal models developed for the study of the disease, in Psoriasis, C. Anca, Editor. London, UKIntechOpen: Rijeka. 2017. p. Ch. 5

[53] Ni X, Lai Y. Keratinocyte: A trigger or an executor of psoriasis? Journal of Leukocyte Biology. 2020;**108**(2):485-491

[54] Bhopale MK et al. DAB(389) IL-2 recombinant fusion toxin effect on lymphocyte- and macrophage-producing cytokine subpopulation cells in experimentally induced demyelinating disease in mice. Immunopharmacology and Immunotoxicology. 2017;**39**(6):318-329

[55] Attia SS et al. A potential role of mesenchymal stem cells derived from human umbilical cord blood in ameliorating psoriasis-like skin lesion in the rats. Folia Morphologica. 2022;**81**(3):614-631

[56] Eaton LH et al. Impaired Langerhans cell migration in psoriasis is due to an altered keratinocyte phenotype induced by interleukin-17. British Journal of Dermatology. 2018;**178**(6):1364-1372

[57] Choi EW et al. Reversal of serologic, immunologic, and histologic dysfunction in mice with systemic lupus erythematosus by long-term serial adipose tissue–derived mesenchymal stem cell transplantation. Arthritis and Rheumatism. 2012;**64**(1):243-253

[58] Prieto MH, et al. New Approaches to Dermatological Conditions Employing Mesenchymal Stem Cell Therapies. Clinical Dermatology Open Access Journal. 2018;**3**(6)

[59] Shin T-H et al. Mesenchymal stem cell therapy for inflammatory skin diseases: Clinical potential and mode of action. International Journal of Molecular Sciences. 2017;**18**(2):244

[60] Anudeep TC et al. Advancing regenerative cellular therapies in non-scarring alopecia. Pharmaceutics. 2022;**14**(3):612

[61] Siam M, Elsheikh AK, Attia N. Alopecia in dogs causes, incidance and clinical signs with special reference to nutritional alopecia. Zagazig Veterinary Journal. 2022;**50**(2):136-147

[62] Bak DH et al. Human umbilical cord blood mesenchymal stem cells engineered to overexpress growth factors accelerate outcomes in hair growth. Korean Journal of Physiology and Pharmacology. 2018;**22**(5):555-566

[63] Salhab O, Khayat L, Alaaeddine N. Stem cell secretome as a mechanism for restoring hair loss due to stress, particularly alopecia areata: Narrative review. Journal of Biomedical Science. 2022;**29**(1):77

[64] Schmidt B, Horsley V. Unravelling hair follicle-adipocyte communication. Experimental Dermatology. 2012;**21**(11):827-830

[65] Jackson WM, Nesti LJ, Tuan RS. Mesenchymal stem cell therapy for attenuation of scar formation during wound healing. Stem Cell Research & Therapy. 2012;**3**(3):20

[66] Millán-Rivero JE et al. Silk fibroin scaffolds seeded with Wharton's jelly mesenchymal stem cells enhance re-epithelialization and reduce formation

of scar tissue after cutaneous wound healing. Stem Cell Research & Therapy. 2019;**10**(1):126

[67] Gentile P. New strategies in plastic surgery: Autologous adipose-derived mesenchymal stem cells contained in fat grafting improves symptomatic scars. FBL. 2021;**26**(8):255-257

[68] An Y-H et al. High-efficient production of adipose-derived stem cell (ADSC) Secretome through maturation process and its non-scarring wound healing applications. Frontiers in Bioengineering and Biotechnology. 2021;**9**:9

[69] Lukiswanto BS et al. Evaluation of wound healing potential of pomegranate (Punica granatum) whole fruit extract on skin burn wound in rats (Rattus norvegicus). Journal of Advanced Veterinary and Animal Research. 2019;**6**(2):202-207

[70] Efimova IO, et al. Treatment of burns All-Russian student scientific-practical conference with participation of pupils of 10-11 128 forms, in Student science - the first step and academic science. In: IOP Conference Series, Earth Environment Science. 2019;**346**:012057

[71] Zhang J et al. Immunosuppressive effects of mesenchymal stem cell transplantation in rat burn models. International Journal of Clinical and Experimental Pathology. 2015;**8**(5):5129-5136

[72] Chen Y-W et al. The effects of adipose-derived stem cell–differentiated adipocytes on skin burn wound healing in rats. Journal of Burn Care & Research. 2017;**38**(1):1-10

[73] Rangatchew F et al. Mesenchymal stem cell therapy of acute thermal burns: A systematic review of the effect on inflammation and wound healing. Burns. 2021;**47**(2):270-294

[74] Alapure BV et al. Accelerate healing of severe burn wounds by mouse bone marrow mesenchymal stem cell-seeded biodegradable hydrogel scaffold synthesized from arginine-based poly(ester amide) and chitosan. Stem Cells and Development. 2018;**27**(23):1605-1620

[75] Hacker S et al. Paracrine factors from irradiated peripheral blood mononuclear cells improve skin regeneration and angiogenesis in a porcine burn model. Scientific Reports. 2016;**6**:25168

[76] Kudinov VA et al. Antimicrobial and regenerative effects of placental multipotent mesenchymal stromal cell Secretome-based chitosan gel on infected burns in rats. Pharmaceuticals (Basel). 2021;**14**(12):17-18