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Chapter

Mesenchymal Stem/Stromal Cells and Fibroblasts: Their Roles in Tissue Injury and Regeneration, and Age-Related Degeneration

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Abstract

Mesenchymal stem/stromal cells (MSCs) and fibroblasts are present in normal tissues to support tissue homeostasis. Both share common pathways and have a number of common features, such as a spindle-shaped morphology, connective tissue localization, and multipotency. In inflammation, a nonspecific response to injury, fibroblasts and MSC are the main players. Two mechanisms of their mode of action have been defined: immunomodulation and regeneration. Following tissue injury, MSCs are activated, and they multiply and differentiate, to mitigate the damage. With aging and, in particular, in degenerative disorders of the musculoskeletal system (i.e., joint and bone disorders), the regenerative capacity of MSCs appears to be lost or diverted into the production of other nonfunctional cell types, such as adipocytes and fibroblasts. Fibroblasts are stromal cells that provide the majority of the structural framework of almost all types of tissues; i.e., the stroma. As such, fibroblasts also have significant roles in tissue development, maintenance, and repair. In their immunosuppressive role, MSCs and fibroblasts contribute to the normal resolution of inflammation that is a prerequisite for successful tissue repair. In this chapter, we review the common and opposing properties of different tissue-derived MSCs and fibroblasts under physiological and pathophysiological conditions. We consider injury and age-related degeneration of various tissues, and also some immunological disorders. Specifically, we address the distinct and common features of both cell types in health and disease, with a focus on human synovial joints. Finally, we also discuss the possible approaches to boost the complementary roles of MSCs and fibroblasts, to promote successful tissue regeneration.

Keywords: Mesenchymal stem/stromal cells, fibroblasts, tissue injury, age-related tissue degeneration, tissue regeneration

1. Introduction

Mesenchymal stem/stromal cells (MSCs) represent tissue-resident progenitor cells with multi-differentiation potential *in vivo* (stem cells) and *in vitro* (stromal cells) [1].

Although MSCs were first described several decades ago [2, 3], their nature, roles, definitions, and even name remain to be fully defined. The largest bone of contention lies in their designation as stem cells. Even Arnold Caplan, who first coined the term 'mesenchymal stem cells' [4], has suggested recently that it is time to change the name, to avoid unprecedented expectations of regrowth of new tissues and organs [5]. About 15 years ago, the International Society for Cellular Therapy set up minimal criteria for the definition of MSCs *in vitro*, which include plastic adherence, trilineage differentiation, and a set of negative and positive markers [6]. These initial efforts were further up-graded as the knowledge of the *in-vitro* properties of MSCs accumulated, in particular for their role in immunomodulation [7].

Great advances have been made in the *in-vivo* identification of human skeletal stem cells (SSCs). Following their identification in mouse bone marrow, Chan et al. unraveled the hierarchy of positive markers (i.e., podoplanin, CD73, CD164) and negative markers (i.e., CD146) of the self-renewing, multipotent human SSCs. These cells can be isolated from human fetal and adult adipose stroma following treatment with bone morphogenetic protein 2, and they can undergo local expansion in response to acute skeletal injury [8]. In addition, the same group recently identified a way to boost the endogenous SSCs to aid in the repair of worn out cartilage in osteoarthritis [9].

In contrast to the huge advances made in the field of bone-marrow-derived MSCs, the identity and role of MSCs resident in other tissues are still largely unknown. Initially, MSCs were believed to be common progenitors of all musculoskeletal tissues. On this basis, several hypotheses on the developmental origins of MSCs were put forward. The pericyte hypothesis, for example, suggested that MSCs are pericytes and are thus common to every vascularized tissue [10]. However, Guimarães-Camboa et al. rejected this theory, and revealed that pericytes do not behave as stem cells during aging and injury [11]. They traced transcription factor Tbx18 (as a selective marker of pericytes and vascular smooth muscle cells) to follow the fate of these cells in aging and in injury models in multiple adult organs. In this way they showed that pericytes maintained their identity through aging and in diverse pathological settings, and hence did not significantly contribute to other cell lineages [11]. Currently, what we do know is that MSCs are tissue-specific progenitors that can differentiate into their tissue of origin [12, 13] and exhibit tissue of origin-specific profiles and response to inflammatory stimuli [14]. Although MSCs have already been used in clinical practice in the form of cell injections for treatment of several degenerative disorders, unfortunately much of their reported anti-aging and regenerative potential remains unsupported [15, 16]. Hence, their potential in regenerative medicine is still largely underexploited.

Fibroblasts are historically even 'older' than MSCs, as they were first described over a century ago [17]. However the criteria for their definition is even more poorly established than that for MSCs [18–20]. Fibroblasts constitute the majority of the cells of the structural framework, or stroma, of almost all types of tissues [20]. Their main role is the secretion of extracellular matrix molecules, such as collagen, proteoglycans, and others. As the different types of collagen are the major component of tissues such as bone, cartilage, and skin, fibroblasts also have significant roles in tissue development, maintenance, and repair. Fibroblasts from different tissues were long considered as functionally homogenous cells, however significant differences in transcriptome, epigenome and function were demonstrated for synovial fibroblasts from different anatomical locations in joints [21]. Under certain conditions, fibroblasts can also transform into more aggressive phenotypes and contribute to disease pathophysiology, such as in cancers and rheumatoid arthritis [22].

Mesenchymal stem/stromal cells and fibroblasts share numerous common features, as has been reviewed elsewhere [20, 23]. As these cells participate in the common pathways of tissue development, maintenance and healing, either working together or in opposition, this chapter provides an overview of recent studies on these

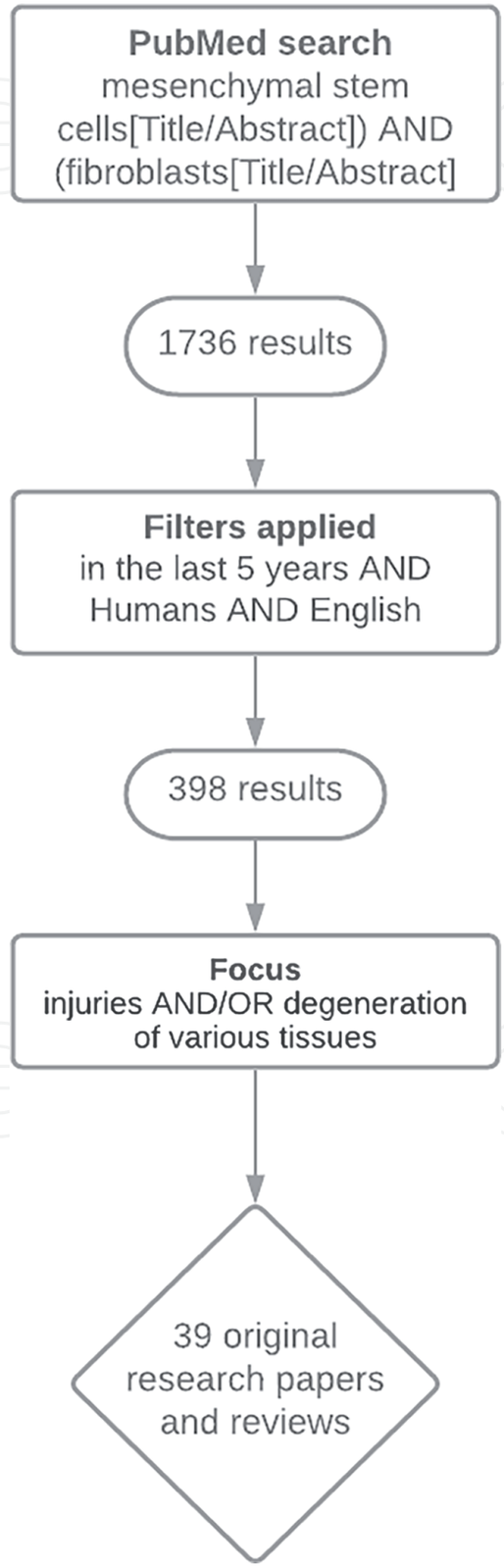

Figure 1.
The approach used to search and select the papers included in this review.

shared and opposing properties of MSCs and fibroblasts with a focus on tissue injury and age-related tissue degeneration, in particular in joint health and disease.

For the purpose of this review, we performed a literature search in PubMed according to the search terms and filters shown in **Figure 1**. To focus on human studies carried out in the past 5 years, we excluded all studies dealing with tumor research, which covers a particularly large research area. We included only those studies dealing with tissue injuries and regeneration, and age-related degeneration. Finally, we also discuss the options for diverting tissue healing processes toward morphological and functional regeneration, rather than the creation of poorly functioning scar tissue to cover such defects.

2. MSCs and fibroblasts in general: their common and distinct properties

A summary of the recent studies that have compared various tissue-derived MSCs and fibroblasts face to face is provided in **Table 1**. A schematic representation of the distinct and common features of MSCs and fibroblasts in health and disease, with a focus on human synovial joints is shown in **Figure 2**.

2.1 Common properties: tissue remodeling and immunomodulation

In contrast to the extremely rare status of MSCs in almost all adult connective tissue (i.e., from 1 to 25 cells per 1,000,000 cells in bone marrow are MSCs [32, 33]), fibroblasts are the most abundant cell type in connective tissue [22]. Fibroblasts are the maintainers of extracellular matrix turnover, and they regulate several physiological processes. In contrast, MSCs are quiescent most of the time, but have self-renewing capacity. However, in response to certain stimuli, such as tissue injury, MSCs respond promptly, resulting in their activation and proliferation, and their differentiation into the terminal cell types that are required for regeneration following an injury [8, 33]. Both cell types can provide the stroma, in particular as collagen for tissues during injury and wound healing. However, it appears that the repair processes that result in formation of a functional tissue, such as collagen type II in cartilage injury, is a feature of MSCs, and particularly for those of the synovium [34]. Fibroblasts or other tissue-derived MSCs (e.g., bone marrow) might be responsible for the filling of defects in cartilage injury with only fibrous tissue; i.e., the fibrocartilage, which is a nonfunctional tissue [9, 35]. Although some early studies showed efficacy for fresh human skin allografts in the treatment of diabetic ulcers, severe burns, and other such injuries, recent studies have instead suggested that fibroblasts are more likely contaminants in such cell therapies, and thus they should be depleted so as not to impede the rejuvenation effects of stem cells [36]. There is also evidence that fibroblasts can undergo aggressive transformation in response to the tumor microenvironment, and thus contribute to disease pathophysiology, such as in cancers [22].

Immunomodulation is a fundamental characteristic of all stroma, which includes, in particular, immunosuppressive effects [37]. Jones et al. showed that stromal cells (e.g., chondrocytes, fibroblasts from synovial joints, lung, skin) can inhibit proliferation of peripheral blood mononuclear cells following polyclonal stimuli. In contrast to parenchymal cells, stromal cells showed antiproliferative functions, irrespective of their differentiation potential and/or content of progenitor cells [37].

Reference	Source of MSCs	Source of fibroblasts	Methods	Findings
[24]	MSCs (Lonza)	Normal dermis	Inflammatory stimulation and subsequent treatment with dexamethasone; multidimensional molecular profiling	Induction of the secretion of cytokines, proteases, and other inflammation agonists and pro- and anti-inflammatory eicosanoids; dexamethasone down-regulated most cytokines and proteases, and pro- and anti-inflammatory eicosanoids; similar profiling for fibroblasts and MSCs
[25]	Thoracic aorta; femoral artery	Dermis (healthy donor); Prostatic stromal myofibroblast cell line (ATTC, CRL-2854)	Immunophenotyping; immunomodulation (PBMC activated with PHA assay); angio-, adipo- and osteo-genesis *in vitro*	All cells expressed CD44 and FSP; similar expression of CD90 and CD105 between MSCs and myofibroblast; dermal fibroblasts completely negative; MSC unique anti-inflammatory and wound healing capacities
[26]	Bone marrow, fat, amnion, chorion, umbilical cord	Three different human skin dermis layers (dermo-hypodermal junction, intermediate reticular dermis, superficial papillary dermis)	Genome-wide transcriptome profiling	Three skin fibroblast types form clearly distinct group from five tissue-derived MSC types
[27]	Bone marrow (ATTC, PCS-500-012)	Dermis (ATTC, PCS-201-012)	Next generation RNA sequencing	Different molecular signatures between MSCs and fibroblasts; homeobox genes with important roles in embryonic development were predominantly expressed in MSCs
[28]	Bone marrow; endometrium and FACS-isolated cells ($PDGFR\beta^+$ and $CD146^+$)	Endometrium and FACS-isolated cells ($PDGFR\beta^+$ and $CD146^-$)	Immunophenotyping; cell proliferation and migration; cytokine/chemokine secretion profiling (+/-LPS)	Both types of MSCs have similar stem cell surface markers, and higher proliferation and migration potential compared to fibroblasts; bone-marrow-derived MSCs showed greater cytokine secretion after stimulation with LPS, in comparison to endometrium-derived MSCs and fibroblasts
[29]	Adipose tissue from discarded material from three different donors undergoing elective surgery	Discarded material from three different donors undergoing elective surgery	Extracellular matrix production *in vitro*; immunofluorescence for collagen type I and fibronectin; ELISA quantification of collagen I	Adipose-derived MSCs produce more fibronectin- and collagen-containing dermal matrix upon stimulation with ascorbic acid, compared to fibroblasts

Reference	Source of MSCs	Source of fibroblasts	Methods	Findings
[30]	Umbilical cord blood-derived MSCs (Medipost Co Ltd.)	Dermis of healthy and diabetic adults	Cell proliferation; collagen and glycosaminoglycan levels	MSC-treated group showed significantly higher collagen synthesis and glycosaminoglycan levels than fibroblast-treated group
[31]	Bone marrow (CD105 and $CD271^{+}$)	Male foreskin	Metabolically and hypoxia conditioned media from MSC and fibroblast migration assays	Both conditioned media have high concentrations of angiogenic factors; fibroblast-derived media attracted MSCs as efficiently as media produced by MSCs

ATTC, American Type Culture Collection; FACS, fluorescence activated cell sorting; LPS, lipopolysaccharide, PBMC, peripheral blood mononuclear cells, PHA, phytohemagglutinin; FSP, fibroblast-specific protein; PDGFRβ, platelet derived growth factor receptor β.

Table 1.
Overview of recent studies with face-to-face comparisons of various tissue-derived MSCs and fibroblasts.

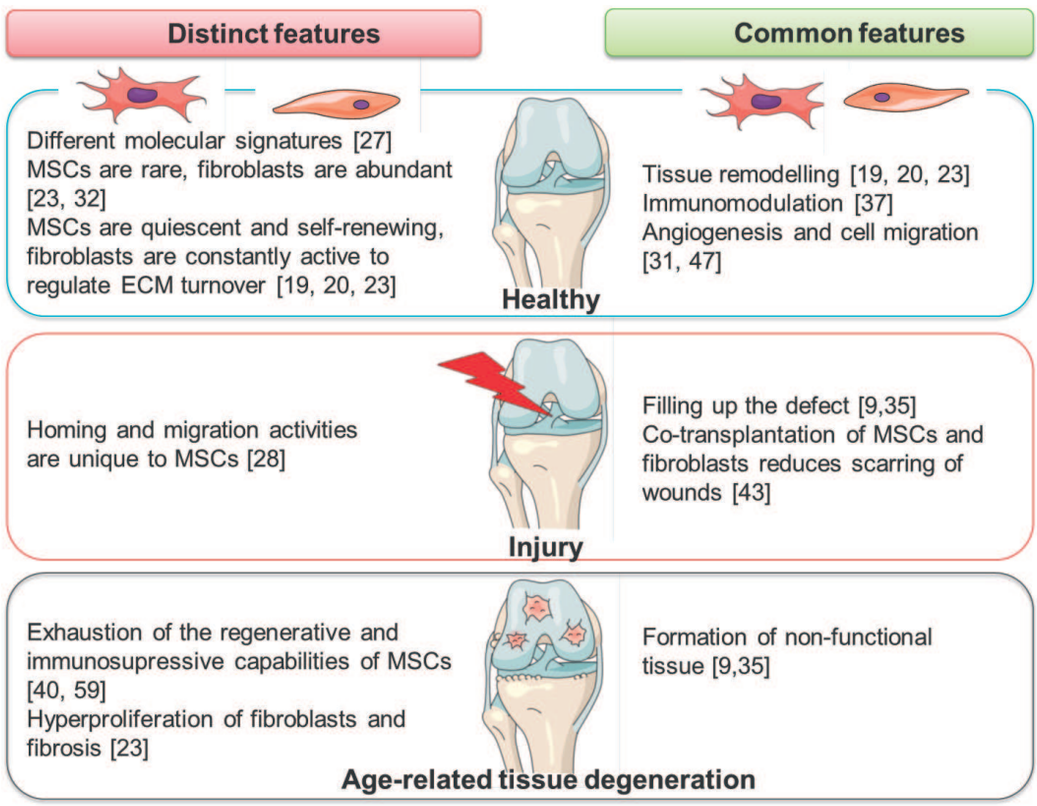

Figure 2.
Schematic representation of the distinct and common features of MSCs and fibroblasts in health and disease, with a focus on human synovial joints. ECM, extracellular matrix.

During inflammation, proteins and lipids secreted by various cells act in a concerted fashion. Tahir et al. analyzed the formation of the most relevant inflammation mediators, including proteins and lipids, in human fibroblasts and MSCs upon inflammatory stimulation and subsequent treatment with dexamethasone [24]. They showed that fibroblasts and MSCs have similar secretion profiles for stimulation and modulation of inflammation [24].

In contrast, there are also studies that have provided evidence of greater anti-inflammatory and wound-healing features of MSCs in comparison to other stromal cells [25]. In an array of *in-vitro* tests to compare human artery-wall-derived MSCs with dermal fibroblasts and myofibroblasts, Pasanisi et al. showed some profound differences in the immunomodulatory properties between these cell types [25]. Both the dermal fibroblasts and myofibroblasts expressed very low levels of immunomodulatory and inflammation-related genes, and had lower immunosuppressive potential for proliferation of peripheral blood mononuclear cells in comparison to the femoral artery MSCs. They also suggested that the two highly sought after translational abilities, as anti-inflammatory and wound healing activities, are unique features of MSCs [25].

Although MSCs and fibroblasts share common sources for their isolation, such as adipose tissue, muscle, and skin, most recent studies have used bone marrow as the source of MSCs and skin as the source of fibroblasts. Following their plastic adherence after isolation and *in-vitro* culture expansion, fibroblasts are morphologically indistinguishable from MSCs, as they both have a spindle-shaped morphology [20]. They also both express the same positive mesenchymal markers, and both lack hematopoietic markers [19]. They also both show trilineage differentiation; i.e., adipogenesis, osteogenesis, and chondrogenesis [36]. Hence, the minimal criteria set by the International Society for Cellular Therapy to define MSCs [6] can also define fibroblasts [20]. Despite great effort, the lack of a specific marker to distinguish between MSCs and fibroblasts represents a major limit in the study of these cells [25].

2.2 Distinct properties: transcriptome profile and migration capacity

Haydont et al. recently performed a wide comparison of skin fibroblasts from three different locations in the deep dermis and hypodermis with five different tissue-derived MSCs [26]. Using genome-wide transcriptome profiling, they showed a clear 'fibroblast' molecular identity that did not segregate with the MSCs. The molecular signature that identified the fibroblasts comprised transcripts associated with hyaluronic acid, aggrecan, collagen processing, collagen fibril anchorage points, the elastic networks, and some others [26]. Similarly, using next-generation RNA sequencing, Taşkiran and Karaosmanoğlu showed that human primary bone marrow MSCs and human primary dermal fibroblasts have different molecular signatures [27]. In particular, a large group of genes that have important roles in embryonic development were highly expressed in MSCs; e.g., the homeobox genes. Aristaless-like homeobox family member ALX1 and distal-less homeobox DXL1, 5, and 6 are involved in craniofacial development, while short stature homeobox (SHOX) regulates expression of early osteogenic genes during cell differentiation. Taşkiran and Karaosmanoğlu suggested that MSCs are more appropriate for developmental and differentiation studies, compared to dermal fibroblasts [27].

Another feature that appears to be more attributed to MSCs is homing through migration. Intrinsic inflammatory characteristics have a pivotal role in stem-cell recruitment [28]. Bone marrow-derived MSCs have been demonstrated to migrate to the endometrium to contribute to the stem-cell reservoir and the regeneration of endometrial tissue [28]. Khatun et al. compared inflammation-driven migration of human bone-marrow-derived MSCs to MSCs and fibroblasts derived from the same niche (i.e., the endometrium). They showed that similar to bone-marrow-derived MSCs, endometrial MSCs showed high migration activity. However, the differentiation process toward stromal fibroblasts resulted in minimal migration [28].

3. MSCs and fibroblasts: their roles in tissue injury

A schematic representation of the interactions between MSCs and fibroblasts is shown in **Figure 3**. Following tissue injury through bone fracture, joint trauma, muscle tears, and skin wounds, for example, a well-orchestrated series of time-dependent and overlapping events takes place, including coagulation, inflammation, new tissue formation, and injury resolution. Each phase needs to be efficiently carried out to allow the further progression toward tissue regeneration.

MSCs can secrete a variety of cytokines and growth factors that have immunosuppressive and antifibrotic properties, which can have beneficial influences in the healing process [38]. The failure of tissue regeneration most commonly results in chronic inflammation and/or fibrosis, which leads to damage of the adjacent tissues and/or formation of inferior nonfunctional tissue. Some tissues have poor healing capacities if a wound extends beyond the epidermis, such as skin and cartilage, in particular. It is not entirely clear whether this is due to the absence or 'exhaustion' of the endogenous MSCs in these tissues, due to disease or age [39, 40]. Fibrosis, or scarring, is defined as accelerated accumulation of extracellular matrix factors, as predominantly collagen type I, which can prevent regeneration of tissue. This can occur in virtually any tissue as a result of trauma, inflammation, immunological rejection, chemical toxicity, or oxidative stress [38]. Following cartilage surface injury, the hyaline cartilage that is predominantly collagen type II is replaced by collagen type I, which lacks the functional properties of cartilage, such as shock absorption and reduction of friction in the joint.

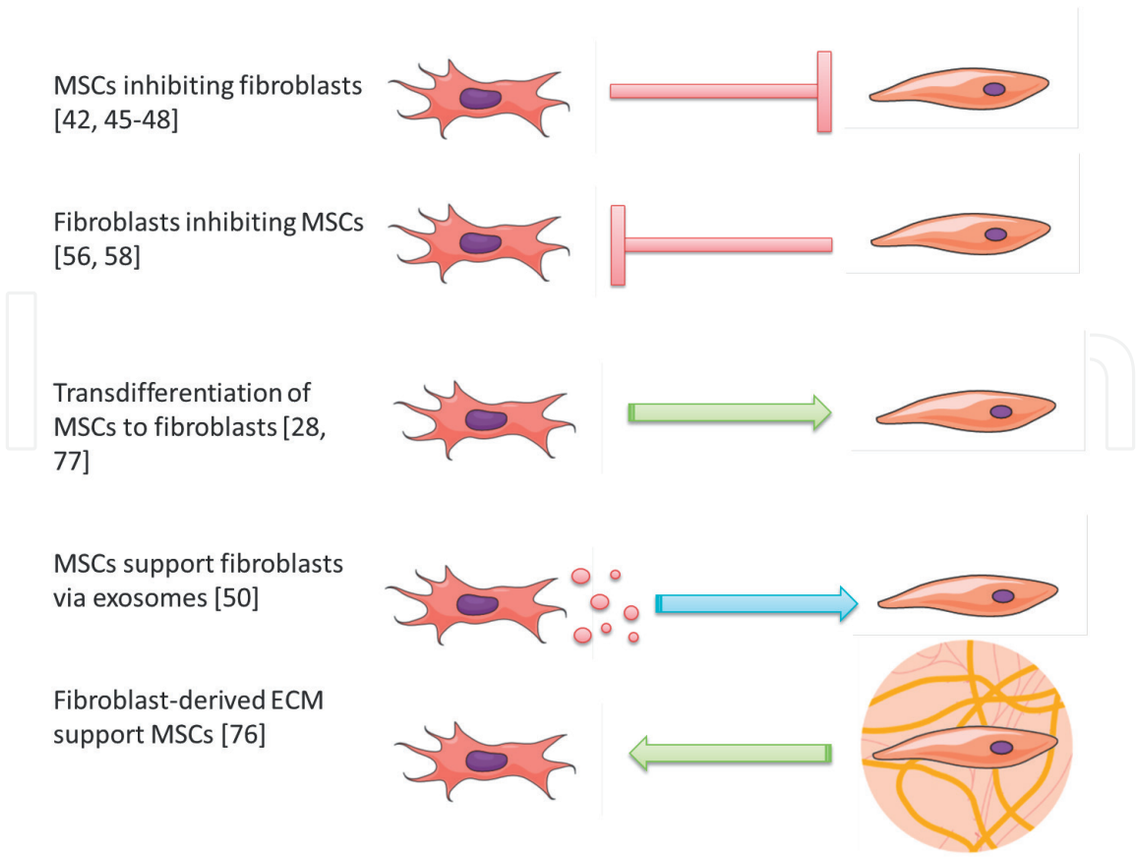

Figure 3.
Schematic representation of the interactions between MSCs and fibroblasts as observed in the in vitro studies. ECM, extracellular matrix.

The antifibrotic effects of MSCs are not entirely understood, and they are likely to overlap with the MSC anti-inflammatory and angiogenic properties [38, 41]. However, MSCs secrete several cytokines and growth factors that inhibit fibroblasts [42]. Hepatocyte growth factor released by MSCs has been shown to down-regulate the expression of transforming growth factor-β1 (TGF-β1) and collagen type I and III by fibroblasts, and on the other hand, to up-regulate expression of matrix metalloproteinases 1, 3, and 13 in fibroblasts, thereby promoting turnover of the extracellular matrix [42]. In agreement with this, Yates et al. showed that co-transplantation of MSCs and fibroblasts reduces scarring of wounds [43]. They transplanted xenogeneic MSCs and showed that these augmented fibroblast proliferation and migration, and the extracellular matrix deposition that is critical for wound closure; this co-transplantation also reduced inflammation following wounding, an effect that was greater than seen for MSCs or fibroblasts alone. These data suggested complementary roles of MSCs and fibroblasts to normalize matrix regeneration during healing, and they demonstrated that even transiently engrafted cells can have a long-term impact via matrix modulation and 'education' of other tissue cells [43].

Domaszewska-Szostek et al. recently reviewed the available data on the efficiency of cell therapies for the treatment of chronic wounds, with these therapies including fibroblasts, keratinocytes, fibroblasts and keratinocytes together, bone-marrow-derived MSCs, and adipose tissue cells [44]. They showed that the majority of reports were on fibroblasts and keratinocytes, which included cell-based products that are already on the market. Based on the knowledge at the time, Domaszewska-Szostek et al. suggested that cell therapies in the treatment of chronic wounds showed immense potential. However, much is yet to be determined from both sides, in terms of both patients and cell therapies [44].

3.1 Skin injuries

While fibroblast-based substitutes have already been used in regenerative medicine, and in particular in regeneration of skin, a recent study by Paganelli et al. suggested that adipose-tissue-derived MSCs might represent a better alternative to fibroblasts in full-thickness skin injuries [29]. They showed that *in-vitro* adipose-tissue-derived MSCs produce a collagen- and fibronectin-containing dermal matrix that is more abundant than for fibroblasts [29]. Moreover, adipose-tissue-derived MSCs also served as modulators in the regeneration of tissue that was inflamed or scarred secondary to injuries such as burns or trauma. Liu et al. investigated the effects of adipose-tissue-derived MSCs on keloidal disease, which is a particular type of scarring that is considered to arise from excessive proliferation of fibroblasts and extracellular matrix deposition [45]. They used a starvation-induced conditioned medium from adipose-tissue-derived MSCs to treat human keloid-derived fibroblasts, and evaluated the fibroblast *in-vitro* proliferation, migration, and apoptosis. These human keloid-derived fibroblasts showed inhibited proliferation and collagen synthesis. They also used a keloid xenograft implantation animal model to assess the paracrine effects of conditioned medium from adipose-tissue-derived MSCs *in vivo*. They noted reduced inflammation and fibrosis in an *in-vivo* keloid model, which was seen as keloid shrinkage and reduced inflammatory cell accumulation, blood vessel density, and collagen deposition [45].

Han et al. took things a step further, and included a photobiomodulation pre-treatment of adipose-derived MSCs before collection of their conditioned medium. Photobiomodulation is a laser treatment that uses low power and energy, but has been

shown to induce positive photobiological processes in cells, such as regulation of cell secretion, and promotion of cell proliferation, differentiation, and migration, with enhanced immunological functions, and therefore, accelerated tissue repair [46]. However, when they cultured hypertrophic scar and keloid fibroblasts in conditioned medium from adipose MSCs pretreated with photobiomodulation therapy for 12, 24, and 48 h, there was inhibition of proliferation of these fibroblasts, and down-regulation of their profibrotic growth factors and collagen synthesis. They also suggested that the mechanism for this inhibition was related to down-regulation of TGF-β1 and Notch-1 expression [46].

In addition to adipose-tissue-derived MSCs, bone-marrow-derived MSC have shown benefits for keloids and hypertrophic scars. Fang et al. showed that bone-marrow-derived MSCs use a paracrine signaling mechanism to attenuate the fibroblast proliferative and profibrotic phenotypes derived from hypertrophic scars and keloids, and to inhibit extracellular matrix synthesis [47]. Using conditioned medium from bone-marrow MSCs, they showed significant inhibition of proliferation and migration of the fibroblasts from hypertrophic scars and keloids, in comparison with the use of conditioned medium from normal skin fibroblasts. Furthermore, they also reported that for conditioned medium from bone-marrow-derived MSCs, for both of these types of fibroblasts, there was decreased expression of profibrotic genes, including those for connective tissue growth factor, plasminogen activator inhibitor-1, TGF-β1, and TGF-β2, and increased expression of antifibrotic genes, including those for TGF-β3 and decorin. Moreover, they reported decreased expression of collagen I and fibronectin and low levels of hydroxyproline in the cell culture supernatant, which suggested that the conditioned medium from bone MSCs suppressed the synthesis of extracellular matrix in these fibroblasts [47].

Similar data were reported by Sato et al. for amnion-derived MSCs. Following harvesting of keloid, mature and normal fibroblasts, and their stimulation with TGF-β, they showed that conditioned medium obtained from the amnion-derived MSCs prevented proliferation and activation of the keloid fibroblasts [48].

Tooi et al. used a similar study design; however, they used conditioned medium from human placenta-derived MSCs to harvest exosomes, and examined their effects on normal adult dermal fibroblasts *in vitro* [49]. Exosomes contain nucleic acids, proteins, and lipids, and function as an intercellular communication vehicle for mediation of the paracrine effects of MSCs [49]. They reported positive effects of this treatment, and in particular, significant up-regulation of stemness-related genes, such as octamer-binding transcription factor 4 (Oct4) and NANOG homebox gene, and differentiation competence of fibroblasts to adipocytes and osteoblasts [49].

Hu et al. investigated the roles of exosomes derived from adipose MSCs in cutaneous wound healing [50]. *In vitro*, they showed that these exosomes can be taken up and internalized by fibroblasts, to stimulate cell migration and proliferation, and collagen synthesis, in a dose-dependent manner. *In vivo*, they demonstrated that these exosomes can be recruited to soft tissue wound areas in a mouse skin incision model, and that they significantly accelerated cutaneous wound healing. Following systemic administration of exosomes, they reported increased collagen I and III production in the early stage of wound healing, and inhibited collagen expression in the late stage, which might be favorable to reduce scar formation. Based on these results, they suggested that exomes can be used to facilitate cutaneous wound healing via optimizing the characteristics of fibroblasts [50].

Li et al. explored the paracrine effects of conditioned medium from umbilical-cord-derived MSCs on dermal fibroblasts [51]. They showed that this treatment

increased the proliferation and migration of fibroblasts. Moreover, they also reported on their transition into a phenotype with a low myofibroblast formation capacity, a decreased ratio of TGF-β1/3, and an increased ratio of matrix metalloproteinase/ tissue inhibitor of metalloproteinases. They also performed *in-vivo* wound healing assays. Full thickness skin excisional wounds treated with conditioned medium from umbilical-cord-derived MSCs showed accelerated healing, with fewer scars seen.

Pan et al. investigated the effects of conditioned medium derived from human amniotic MSCs on hydrogen-peroxide-induced senescence of human dermal fibroblasts. They showed that the conditioned medium derived from these cells significantly decreased senescence-associated β-galactosidase activity, and promoted proliferation of senescent human dermal fibroblasts [52]. Interestingly, they also showed the same effect using conditioned medium from human amniotic epithelial cells. These cells were isolated from the same amniotic tissue, and characterized by their similar immunophenotype to the MSCs, except for stage-specific embryonic antigen-4 as specific to MSCs, and their cobblestone-like morphology, in contrast to the MSC fibroblast morphology [52].

Gabrielyan et al. directly compared metabolically conditioned medium and hypoxia-conditioned medium derived from bone-marrow MSCs and skin fibroblasts, and evaluated their attraction of bone-marrow MSCs in two-dimensional migration assays [31]. They reported that the conditioned media from both types of cells had high concentrations of the angiogenic factors that are important for angiogenesis and cell migration. Having shown that both of the conditioned media produced by human skin fibroblasts attracted MSCs as efficiently as conditioned medium produced by human bone-marrow MSCs, these authors favored fibroblasts-derived metabolic conditioning as providing easier, cheaper, and faster access to chemoattractive agents [31].

3.2 Diabetic wounds

There are also several studies that have suggested superior effects of MSCs compared to fibroblasts for the stimulation of diabetic wound healing [30, 53]. Jung et al. compared the treatment effects of human umbilical-cord-blood-derived MSCs with those of fibroblasts on diabetic wound healing *in vitro* [30]. Using co-culture of diabetic fibroblasts with either healthy fibroblasts or umbilical-cord-blood-derived MSCs over 3 days, they measured cell proliferation and collagen synthesis and glycosaminoglycan levels, which are the major contributing factors to wound healing. The group treated with the umbilical-cord-blood-derived MSCs showed significantly greater collagen synthesis and glycosaminoglycan levels than the fibroblast-treated group [30]. Saheli et al. also focused on the interplay between MSCs and fibroblasts in diabetic wound healing, in both *in-vivo* and *in-vitro* diabetic models [53]. *In vivo*, in the group of diabetic wounds treated with MSC-derived conditioned medium, they demonstrated significantly greater wound closure, less pronounced inflammatory responses in the granulation tissue, better tissue remodeling, and more vascularization, compared with the nontreated diabetic wounds [53]. *In vitro*, they cultured human dermal fibroblasts in a high-glucose medium. When these fibroblasts were incubated in the presence of MSC-derived conditioned medium, they showed up-regulation of the genes encoding epidermal growth factor and basic fibroblast growth factor (bFGF), in addition to significantly greater cell viability/ proliferation, and migration. Based on these findings, they suggested that MSC-derived conditioned medium improves the activity of the fibroblasts in the diabetic microenvironment, and thus might promote wound repair and skin regeneration [53].

3.3 Ligament injuries

Similar to cartilage, ligaments have poor healing capacity due to hypocellularity and lack of cellular components for self-regeneration. Li et al. investigated differentiation of human amnion-derived MSCs into human anterior cruciate ligament fibroblasts *in vitro* using a Transwell co-culture system and induction with bFGF and TGF-β1 [54]. Following an array of gene and protein expression for ligament-specific molecules, they suggested Transwell co-cultures as an optimal system for differentiation of amnion-derived MSCs into ligament fibroblasts [54].

3.4 Periodontal disease and jaw injuries

Osteoradionecrosis of the jaw is a severe chronic adverse effect of ionizing radiation therapy to the head and neck region. It is manifested as soft tissue fibrosis, chronic inflammation of the bone, and osteonecrosis of the maxillofacial region, with histopathological formation phases that are very similar to those of chronic wounds [55]. Zhuang and Zou reported inhibitory effects of irradiation-activated-gingival fibroblasts on osteogenic differentiation of human bone-derived MSCs [56]. They showed that exosome-mediated delivery of miR-23a from irradiation-activated fibroblasts inhibited osteogenesis of bone MSCs via directly targeting C-X-C motif chemokine ligand 12 (CXCL12) [56]. Under this pathological condition, rather than working hand in hand, fibroblasts and MSCs appeared to be on opposing sides of the tissue healing process.

A similar situation has been reported for periodontal diseases. These encompass a wide variety of chronic inflammatory conditions in the gingiva (i.e., soft tissue surrounding the teeth) and the periodontal connective tissues, such as the bone and ligaments [57]. Periodontal disease begins with gingivitis, as localized inflammation of the gingiva that is initiated by bacteria in the dental plaque. If untreated, gingivitis can progress to loss of the gingiva, bone and ligaments, which creates the deep periodontal 'pockets' that are a hallmark of this disease, and which can eventually lead to tooth loss [57]. Periodontal ligaments have MSCs that can form fibroblasts, cementoblasts, and osteoblasts, and can thus be used for periodontal regenerative therapy. However, the fate of their differentiation is under the control of the periodontal cells, either via direct contact or via secretion of humoral factors. Kaneda-Ikeda et al. clarified the regulatory mechanism for MSC differentiation by humoral factors from gingival fibroblasts [58]. They indirectly co-cultured human ilium-derived MSCs with human gingival fibroblasts under osteogenic or growth conditions. Interestingly, they reported that humoral factors released by gingival fibroblasts suppressed osteogenesis of MSCs. This effect was regulated by miRNAs and undifferentiated MSC markers [58].

4. MSCs and fibroblasts: their roles in age-related tissue degeneration

With aging, and in particular with degenerative disorders of the musculoskeletal system such as osteoarthritis and osteoporosis, MSCs appear to be 'exhausted', with a lack of regenerative potential [33, 40, 59], or their regenerative potential is diverted from functional to production of nonfunctional cell types, such as adipocytes and fibroblasts [60, 61]. Fibroblasts, on the other hand undergo hyperproliferation resulting in age-related fibrosis of many tissues and organs, in particularly skin, lung, kidney, liver and heart [23].

4.1 Intravertebral disc degeneration

Degeneration of the intervertebral discs is strongly implicated as a cause of lower back pain, which has been shown to affect up to 85% of people at some point during their lives [62]. Although it is most commonly manifested in adulthood and its progression is closely linked to aging, changes in the cellular microenvironment of the discs can begin as early as a few years after birth [62]. Inflammation has been correlated with degenerative disc disease, but its role in discogenic pain and hernia regression remains controversial. Inflammatory responses might be involved in the onset of the disease, although it is also crucial for maintenance of tissue homeostasis [63].

Clinical studies that have used autologous or allogeneic MSCs to treat patients with back pain have reported some encouraging results [64]. There is also evidence that fibroblasts injected into the degenerated discs remain viable, and thus might represent an effective therapy for prevention or for delay of degenerative diseases of the discs. However these data were obtained in animal models only [65].

Shi et al. showed that transplantation of human dermal fibroblasts into degenerating intervertebral discs of rabbits can significantly increase the markers of disc regeneration (e.g., disc height, collagen type I and II gene expression, proteoglycan content). In comparison to transplantation of rabbit dermal fibroblasts, these results showed similar regenerative trends, but these trends did not reach significant difference. This study also showed that the human cells transplanted into rabbit discs did not induce immune response in the rabbit cells [66].

4.2 Bone degeneration

In addition to disc degeneration, most elderly people develop bone loss with age [54]. The most common clinical manifestation of bone loss is osteoporosis associated with an increased risk of fractures, which can also lead to death. In 2017, new fragility fractures in the EU6 were estimated at 2.7 million, with an associated annual cost of €37.5 billion and a loss of 1.0 million quality-adjusted life years [67]. As osteoblasts have a central role in the process of bone formation, the direct reprogramming of fibroblasts into osteoblasts might be a new way to treat bone fractures in elderly individuals. Chang et al. recently reviewed a large body of literature and proposed several clinical applications of a direct conversion method for generating osteoblasts in patients [68]. Successful direct conversion of fibroblasts into osteoblasts was reported previously in 2015, using defined transcription factors, such as Osterix, runt-related transcription factor 2 (Runx2), Oct3/4, and L-myc [69]. Despite this, Chang et al. concluded that more work is needed to determine the best way to directly reprogram somatic cells into osteoblasts for optimal clinical use. They also suggested that in addition to successful fibroblast-to-osteoblast conversion, future studies will need to consider the optimal cellular microenvironment to promote osteoblast survival and bone formation in patients [68]. The microenvironment is a common component and factor with immense importance for efficacy of cell therapies of any kind [70].

5. MSCs and fibroblasts: their roles in immunological disorders

5.1 Rheumatoid arthritis

Under normal conditions, the joint membrane, i.e. synovium represent the site of the two closely related cell types: i.e., fibroblast-like synoviocytes and synovial MSCs.

These can work hand in hand as immunomodulatory cells to control the magnitude of immune responses. Rheumatoid arthritis is a chronic autoimmune disease that manifests as polyarthritis with joint destruction [71]. The main pathological characteristic of this rheumatic disease is increased proliferation of fibroblasts and accumulation of inflammatory cells, which results in the formation of the 'pannus'. Interestingly, based on the evidence from animal models, Matsuo et al. suggested that resident fibroblasts account for the pathology of rheumatoid arthritis, and not bone-marrow-derived and circulating cells [71]. In addition, genetic lineage tracing studies have suggested that fibroblasts in rheumatoid arthritis originate from local proliferation of resident fibroblasts, differentiation of pericytes and MSCs, and transition of endothelial cells [71]. The main targets in this disease are thus inflammatory cytokines and leukocytes. As MSCs are immunosuppressive, they have great potential in therapies for this inflammatory disease [72]. However, it appears that the swamping of the microenvironment in rheumatoid arthritis with inflammatory cells and cytokines causes loss of efficacy in the responses of the endogenous joint-resident MSCs to the exaggerated immune response. In addition, synovial fibroblasts are likely to derive from synovial-membrane-derived MSCs, which can also to give rise to fibroblast-like synoviocytes, as key players in perpetuation of joint inflammation and destruction in rheumatoid arthritis [73].

5.2 Systemic sclerosis

Systemic sclerosis is a rare autoimmune rheumatic disease that is characterized by excessive production and accumulation of collagen in different tissues. The physiopathology of systemic sclerosis has still not been completely elucidated, although roles for fibroblasts, endothelial cells, immune cells, and oxidative stress have been demonstrated [74]. Several studies have established the beneficial effects of administration of MSCs from various tissue sources in different preclinical models that are characterized by local or systemic fibrosis. Clinical studies are, however, still falling behind. On the other hand, MSCs from patients with systemic sclerosis have been shown to constitutively express factors that stimulate fibrotic and angiogenic processes. This might indicate that MSCs are altered by the environment secondary to the onset of the disease, or that they might participate in the physiopathology of the disease [75]. Hence, the rationale for using allogenic MSCs in systemic sclerosis (as well as in other autoimmune diseases) is based on the possibility that autologous MSCs will be altered in these diseases [74].

6. MSCs and fibroblasts: how to boost their complementary tissue regeneration

6.1 In-vitro *approaches*

As MSCs represent rare cell populations *in vivo*, their *in-vitro* expansion is an often-unavoidable step in the preparation for these cell therapies. Currently, MSC expansion is most commonly achieved via cultivation on tissue culture plastics with the addition of 10% fetal bovine serum. Van et al. investigated the feasibility of human fibroblast-derived extracellular matrix as an alternative for *in-vitro* cell expansion [76]. Such fibroblast-derived extracellular matrix was obtained from decellularized extracellular matrix derived from *in-vitro*-cultured human lung fibroblasts.

Using umbilical-cord-blood-derived MSCs, they directly compared cell cultivation on tissue culture plastics, fibronectin-coated tissue culture plastics, and human fibroblast-derived extracellular matrix. They showed that the last of these, the human fibroblast-derived extracellular matrix, improved cell proliferation, migration, and osteogenesis, as well as the expression of stemness and engraftment-related markers of MSCs. Furthermore, they showed superior *in-vivo* effects of MSCs pre-conditioned on human fibroblast-derived matrix in an emphysema animal model (i.e., a lung disease). Based on this, they suggested that human fibroblast-derived matrix represents a naturally derived biomimetic microenvironment with potential for practical applications in regenerative medicine [76].

Adipose-derived MSCs represent the preferable autologous source of MSCs in regenerative medicine in general, due to their indispensability in adults. Sivan et al. standardized their *in-vitro* culture conditions for differentiation of adipose-derived MSCs into dermal-like fibroblasts, which can synthesize extracellular matrix proteins [77]. Given that adipose-derived MSCs are multipotent in nature and might develop into undesirable tissues upon transplantation, the diverting of these MSCs to a more committed, fibroblast lineage appears like a better option in skin tissue engineering. To promote commitment of these MSCs into fibroblasts, they used a special biomimetic matrix composite that was pre-coated with fibrinogen, fibronectin, gelatin, hyaluronic acid, and human platelet growth factors. When MSCs were cultured on this composite with the presence of differentiation medium supplemented with fibroblast-conditioned medium and growth factors, they showed up-regulation of fibroblast-specific protein-1 and a panel of extracellular matrix molecules that were specific to the dermis, such as fibrillin-1, collagen I, collagen IV, and elastin. As fibroblasts derived from adipose MSCs can synthesize elastin, this is an added advantage for successful skin tissue engineering, compared to fibroblasts from skin biopsies [77].

To boost the combined tissue-healing effects of MSCs and fibroblasts, several tissue engineering approaches are being investigated. To enhance resistance to oxidative stress and the paracrine potential of MSCs, Costa et al. formulated MSC spheroids encapsulated in alginate microbeads [78]. This three-dimensional formulation showed increased angiogenic and chemotactic potential relative to encapsulated single cells. As the encapsulated MSCs promoted formation of tube-like structures and migration of fibroblasts into the wounded area, these authors suggested that such a model setting can be used for wound repair and regeneration processes [78].

As oxygen represents an important factor in tissue healing, hyperbaric oxygen therapy is an effective adjunct treatment for ischemic disorders, such as chronic wounds. Engel et al. showed beneficial effects of hyperbaric oxygen therapy on mono-cultures and co-cultures of human adipose-derived MSCs and fibroblasts [79]. The results of this study suggested that hyperbaric oxygen therapy leads to immunomodulatory and proangiogenetic effects in a wound-like environment, where adipose-derived MSCs and fibroblasts collaborated toward efficient wound healing [79].

In addition to cell therapies where formulation for clinical use still represents immense challenges, great hope has also been put into the cell-free formulations for use in regenerative medicine. Several studies have explored the effects of conditioned media from various tissue-derived MSCs on fibroblasts (as described in 3.1). Conditioned medium is a cell-free formulation, and it basically defines the adult stem-cell secretome. The majority of studies that used conditioned medium to enhance fibroblast properties, harvested the medium from two-dimensional cultures of MSCs from various tissue sources. Using a polystyrene scaffold, Kim et al. created a three-dimensional culture

of perivascular cells, which represented a more physiologically appropriate system to harvest conditioned medium [80]. They used this medium to investigate the effects on the migration and proliferation of human keratinocytes and fibroblasts. The migration of both of these types of cells, and also the proliferation of keratinocytes, were significantly greater with the conditioned medium from this three-dimensional culture system. They also reported greater expression of type I collagen, specific expression of some other factors (e.g., thioredoxin), and more small particles such as CD63-positive extracellular vesicles, which were shown to stimulate keratinocyte migration. Based on these data, the three-dimensional cultures have the potential to be considered as future wound-healing remedies.

An *in-vivo* alternative to conditioned medium produced by *in-vitro* cultured MSCs was tested by Cerny et al. [81]. They used wound fluid samples from fingertip injuries and split skin donor sites under occlusive dressings, to evaluate the effects of paracrine factors in the wound fluid (secretome) on migration and proliferation of MSCs and fibroblasts. Under these conditions, MSCs showed significant increases in both migration and proliferation, while fibroblasts showed a significant increase in migration only. Hence, the paracrine factors in the wound fluid can modulate the wound-healing process, and can reduce scar-tissue formation [81].

6.2 In-vivo *approaches*

When it comes to *in-vivo* approaches to stimulate endogenous MSCs and fibroblasts, platelet-rich plasma has been widely studied and is used in clinical practice. Platelet-rich plasma contains higher concentrations of platelets than whole blood, as typically three-fold to five-fold higher compared with normal plasma (normal: 150,000 to 300,000 platelets per microliter) [82]. This platelet concentrate has been shown to have anti-inflammatory effects through growth factors, such as TGF-β and insulin-like growth factor 1, and also stimulatory effects on MSCs and fibroblasts [82].

Stessuk et al. evaluated the combined effects of platelet-rich plasma and conditioned medium from adipose-derived MSCs on fibroblasts and keratinocytes *in vitro*. They showed significant proliferation of both cell types, and also significant migration of fibroblasts treated with both components, which suggested the potential of this combination for healing and re-epithelialization of chronic wounds *in vivo* [83].

The major issue of unpredictable and difficult-to-replicate *in-vivo* effects of MSC therapies is most probably the microenvironment that these cell injections are delivered into. In healthy tissues, stem cells reside within a complex microenvironment that comprises cellular, structural, and signaling cues that collectively maintain stemness and modulate tissue homeostasis [70]. Following tissue injury, substantial changes are made to this unique cell environment, which will influence the regulation of stem-cell differentiation, trophic signaling, and tissue healing. Bogdanowicz and Lu reviewed recent studies on how microenvironmental cues modulate MSC responses following connective tissue injury, and how this microenvironment can be programmed for stem-cell-guided tissue regeneration [70]. Based on their revised data, these authors suggested that the cell microenvironment should be conducive to stem-cell lineage commitment, biomimetic tissue regeneration, and ultimately, restoration of physiological functions. In this light, specific attention should be directed to methods for standardization of experimental conditions both *in vitro* and *in vivo*, and in particular to optimization of cell seeding densities and cell sources [70].

To mimic the optimal microenvironment for MSCs, several novel technological approaches are being developed. Combining human fibroblast-derived matrix and the biocompatible polymer hydrogel (i.e., polyvinyl alcohol), Ha et al. demonstrated cytocompatibility with human MSCs [84]. Moreover, this advanced wound healing therapy was shown to be efficient in full-thickness wound repair in a preclinical model [84].

6.3 Converting fibroblasts to MSCs

When it comes to vascular damage, vascular-wall-derived MSCs might be particularly well suited for resolution of such injuries. Recently, Steens et al. developed a method for direct conversion of human skin fibroblasts into vascular MSCs. They directed cell-fate conversion through induction of ectopic expression of the highly vascular MSC-specific *HOX* genes, including HOXB7, HOXC6, and HOXC8, while bypassing pluripotency. The converted MSCs showed classical multipotent MSC characteristics *in vitro* (i.e., multipotency, clonogenicity), and were selectively associated with vascular structures *in vivo*. With respect to their therapeutic potential, these cells suppressed lymphocyte proliferation *in vitro*, while in a mouse model of radiation-induced pneumopathy *in vivo*, they protected the mice against vascular damage, as also for *ex-vivo* cultured human lung tissue [85]. These data suggested an efficient strategy for treatment of vascular diseases, such as hypertension, ischemic diseases, vascular lesions, and others.

In addition to genetic manipulation to convert fibroblasts to MSCs, there is also a chemical method available to convert primary human dermal fibroblasts into multipotent, induced MSC-like cells. Using a defined cocktail of small molecules and growth factors, (six chemical inhibitors, plus TGF-β, bFGF, and leukemia inhibitory factor), Lai et al. converted human fibroblasts into inducible MSCs in a monolayer culture over 6 days, with 38% conversion rate [86]. The inducible MSCs behaved like primary bone-marrow-derived MSCs in terms of their multipotency, clonogenicity, molecular signatures, and surface marker expression profile. Moreover, these MSCs were as effectively as bone-marrow-derived MSCs in their significant protection against fatality with endotoxin-induced acute lung injury in a mouse model. Based on these data, the authors suggested that this chemical conversion of fibroblasts to MSCs is superior to the genetic approach, as this latter might have the risk of insertional mutagenesis [86].

7. Conclusions

The relative failure of decades-long endeavors to establish a clear definition for both MSCs and fibroblasts appears to be a result of the complementary and overlapping roles these cells have in cell homeostasis and tissue development and injury. Indeed, due to the similarities in their morphologies, immunophenotypes, and connective tissue stroma formation, MSCs and fibroblasts are indistinguishable in most *in-vitro* settings. However, *in-vivo* studies, and in particular recent studies using modern analytics such as next-generation sequencing, have indicated that a line can be drawn to distinguish between MSCs and fibroblasts. On the other hand, several studies have demonstrated that it is the cellular therapies that combine both of these cell types that represent the optimal approach for future development of tissue-regenerating strategies.

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Conflicts of interest

The author declares that there are no conflicts of interest.

Nomenclature

bFGF	basic fibroblast growth factor
CXCL12	C-X-C motif chemokine ligand 12
MSCs	mesenchymal stem/stromal cells
OCT-4	octamer-binding transcription factor 4
SHOX	short stature homeobox
TGF-β1	transforming growth factor β1

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