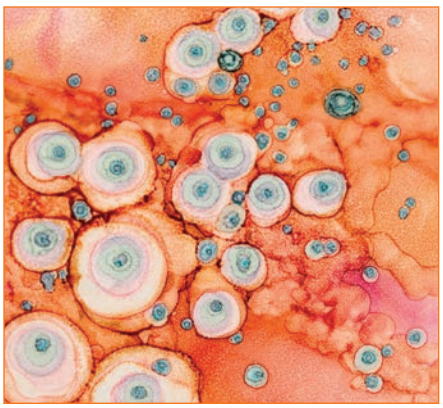

REVIEW

The Safe and Efficacious Use of Secretome From Fibroblasts and Adipose-derived (but not Bone Marrow-derived) Mesenchymal Stem Cells for Skin Therapeutics

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ABSTRACT

Stem cell-based products are rapidly emerging in the marketplace as topical skin care and wound care products. Confusion is prevalent among healthcare providers and end-users about these products. Adipose-derived stem cells, fibroblasts, platelets, and bone marrow-derived stem cells are the most common cells used for stem cell therapeutic development, medical procedures, and skin care products. In this review, the significant advantages of adipose-derived stem cells and fibroblasts in terms of safety and efficacy are highlighted and compared to relatively risky platelets and bone marrow stem cells.
KEYWORDS: Cancer, fibroblasts, inflammation, platelets, secretome, stem cells

Skin care and wound healing products with varying ingredients abound in the marketplace. Stem cells and the molecules that they express or release are rapidly emerging in the skin and wound care markets, often with little or no regulatory oversight, even when injected into patients.[1] Confusion exists among healthcare providers and end-users about the stem cell types, their molecules, and the use of both cells and molecules in formulating topical and injectable products.

Efficacious topical products can be a great preventative strategy for aging skin and its associated afflictions,[2] while wound healing has a great need for efficacious products given the prevalence, costs, and ultimate burden to patients where even death often results from chronic diabetic ulcers.[3] Wound complications can be costly. For example, infection can occur if wounds are open. Therefore, treatments that close wounds quickly and for an extended time are sought, and stem cells offer an important therapeutic strategy for achieving these goals. Therapeutic development, medical procedures, and skin care products on the market currently use a variety of cell types, and the molecules that they express and secrete offer efficacy in a number of indications, including those associated with chronological aging. The environmental, cellular, and molecular mechanisms underlying tissue repair and its failure to heal, or heal without fibrosis, are still poorly understood. Although current therapies are limited given our incomplete understanding of tissue repair, progress has nonetheless been significant for this complicated process of wound healing and a number of successful products have emerged. Stem cell types, including the recently discovered dedifferentiated somatic Gata6 cells in the epidermis,[4] play critical roles in wound healing. In this review, the first of several describing different cell types used for wound healing, I describe some of the most important and widely used cells used for therapeutic development, medical procedures, and skin care products. This first review highlights the safety and efficacy advantages that adipose-derived stem cells and fibroblasts offer compared to relatively risky platelets and bone marrow-derived stem cells. I will not address in detail induced pluripotent stem cells (iPS), used by one company (RxGenesys; Miami, Florida), given that these cells are not ready for therapeutic development.[5] Because of genetic and epigenetic reprogramming errors in these cells,[6,7] a number of resulting abnormalities occur, including their secretomes inducing cancer and fibrosis.[8] While this review specifically highlights stem cell-based therapies for skin, much of what is reviewed here will pertain to healing other tissues.

FIBROBLASTS

Mesenchymal stem cells and fibroblasts produce extracellular matrix (ECM), regulate inflammation and the immune system,

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mediate reparative processes, and serve as pluripotent mesenchymal cells for the generation of multiple cell types in the skin during maintenance and healing processes. Fibroblasts are responsible for expressing, releasing, and depositing ECM molecules, such as several types of collagen, allowing for other cells to migrate along a three-dimensional ECM scaffold to generate the architecture of the skin. Simple ECM topology constructed by the fibroblasts is important because mesenchymal stem cells residing in the ECM will likely alter their paracrine secretion profile depending on the topology; some topologies, presumably ones associated with deregulated ECM, can elicit proinflammatory cytokines such as interleukin (IL)-6.[9] Further, breakdown products of the ECM, including hyaluronic acid (HA) fragments below 800 kDa (with some exceptions) will lead to classically activated macrophages, whereas larger HA molecules induce the anti-inflammatory M2 macrophage phenotype,[10] with maximal specific binding at the CD44 receptor occurring at about 1,000 kDa.[11] Fibroblasts release HA that is attached to exosomes, part of a glycocalyx that surrounds the exosome,[12] and incorporates into and helps to build the ECM.[13] Additionally, fibroblasts produce bioactive molecules that are involved in several physiological processes, including angiogenesis and tissue repair.[14] Clinical trials have demonstrated the effectiveness of fibroblasts to heal chronic venous leg ulcers[15] and dystrophic epidermolysis bullosa,[16] while the secretome from fibroblasts has been used in an acellular collagen hydrogel to heal full-thickness skin wounds in a mouse model.[17] Fibroblasts have been shown to heal an animal model of epidermolysis bullosa, whereas bone marrow stem cells (BMSCs) failed to do so.[18] The secretome from fibroblasts has long been studied and used in the market to successfully treat aged and photodamaged skin as a daily use topical product.[19]

Although the principal cells of connective tissue are called fibroblasts, this is a classification for many cell phenotypes.[20] Tissue-specific environments are generated by stromal cells[21] and differing phenotypes of the fibroblasts can produce either regeneration or fibrosis depending on the need of the tissue that has been wounded. The phenotype of fibroblasts is under the control of many parameters, including mesenchymal stem cells that direct the fibroblast's secretion and placement of ECM.[22] Indeed, this is likely a key mechanism in the ability of adipose-derived mesenchymal stem cells (AMSCs) to induce scar-free wound healing.[22,23] When fibroblasts are cultured with the exosomes from adipose-derived stem cells (ADSCs), the concentration of exosomes was shown to be proportional to the proliferation rate of fibroblasts and the expression of N-cadherin (cyclin-1; PCNA). Further, at an exosome concentration of 50µg/mL, the expressions of collagen III and collagen I were enhanced in the fibroblasts, suggesting an important mechanism by which exosomes secreted from ADSCs optimize a fibroblast phenotype for wound healing.[24] As another phenotype capable of sensing adipokines released by adipocytes, dermal fibroblasts that express receptors for adiponectin and leptin will significantly increase their production of HA and collagen, major ECM components of the dermis. Other phenotypes of dermal fibroblasts secrete matrix metalloproteinases (MMPs) and might therefore exert an antifibrotic effect.[25]

Tissue resident fibroblasts represent a discrete proportion of cells that reside in a given organ and are mostly quiescent or resting cells that are capable of responding to extrinsic cues, including growth factors, cytokines, and mechanical stress, to become activated.[26] The parenchymal injury associated with a nascent and growing tumor is an example of such a cue that can lead to the activation of normal fibroblasts, thereby giving rise, at least in part, to the cancer-associated fibroblasts (CAF) expanding in the tumor.[27] The conversion of normal fibroblasts to the CAF phenotype has been shown to be induced by the secretome of BMSCs.[28] However, even in CAF cells, the outcome of the secretome might limit cancer progression, especially if the tumor environment is normalized.[29] In aged skin, protein secretion by papillary fibroblasts was significantly altered by aging,[30] and a senescent fibroblast phenotype, called the senescence-associated secretory phenotype (SASP), develops that is associated with epithelial proliferation and tumorigenesis[31] and with melasma pathophysiology.[32] Aged fibroblasts, exacerbated by a high-fat diet for example, not only reduce their expression of genes involved in ECM formation, but also gain traits similar to neonatal proadipogenic fibroblasts.[33] To remediate the aberrant secretome from fibroblasts in aging skin, the topical use of a secretome from fibroblasts with a nonsenescent phenotype has been successfully used to treat aged skin and reduce hyperpigmentation.[34]

PLATELET-RICH PLASMA (PRP)

In-vitro mesenchymal stem cell (MSC) expansion media can affect the cell's phenotype. Platelet lysate supplemented media, as used in PRP procedures, favors a proinflammatory MSC phenotype and the secretion of granulocyte macrophage-colony stimulating factor (GM-CSF), which might enhance immune cell recruitment and the maintenance of macrophages in an M1, prototypical inflammatory phenotype.[35] Classically activated M1 macrophages are considered the proinflammatory subtype, whereas alternatively activated, M2 macrophages are known to possess anti-inflammatory properties.[36] Although most platelet interactions with other cell types are restricted to within the blood vessels, outside of the blood vessels, platelets colocalize with macrophages in several models for cutaneous inflammation, where they suppress the expression of anti-inflammatory markers and enhance the synthesis of proinflammatory mediators in the macrophages with which they interact.[37] Therefore, as a proinflammatory procedure, PRP is not generally recommended for therapeutic development. However, if PRP is used for a procedure, follow-on treatment with the secretome from ADSCs can be used because of its proresolving effects (see below).

BMSCs

Blood enters a tissue because of a significant need to close the wound quickly and fight foreign invaders, with BMSCs entering the wound to facilitate the initial phase of wound healing by accelerating rapid closure of the wound[38]; a proinflammatory response also ensues.[39] The recruitment of blood, including BMSCs[40,41] and monocytes, to build a cellular and chemically mediated cytotoxic wall, is distinctly different from the M2-mediated anti-inflammatory response mediated locally in the skin and used to build a cellular wall against foreign invasion.[39] Neutrophils are usually the first leukocytes to arrive at the site of inflammation.[42] Recruited neutrophils mediate acute inflammation through the release of lytic enzymes from their granules, producing reactive

oxygen intermediates that are critical for the clearance of invading bacteria. BMSCs help to maintain the viability and activity of neutrophils by prolonging their survival and function, thus prolonging and enhancing the inflammation.[43]

Macrophages are innate immune cells resident in the skin and are an important part of the early inflammatory response,[44,45] where hypoxia decreases macrophage polarization from the proinflammatory M1 to the anti-inflammatory M2 phenotype by BMSCs, needed to promote wound healing. Toll-like receptor 4 (TLR4)-primed BMSCs mostly secrete proinflammatory mediators (BMSC1 phenotype), while Toll-like receptor 3 (TLR3)-primed BMSCs (BMSC2 phenotype) express mostly immunosuppressive molecules.[46] Hypoxia is known to trigger TLR-4 signaling and induce inflammation.[47] Thus, the local injury environment, where blood-borne BMSCs infiltrate a wound in hypoxic conditions, must be taken into account when evaluating the therapeutic potential of BMSCs, where, in skin injuries, they will not induce an anti-inflammatory M2 macrophage phenotype. Likewise, BMSCs cultured in hypoxic conditions (BMSC1 phenotype) will secrete proinflammatory molecules,[45] and the BMSC2 phenotype is procancerous in both *in-vitro* and *in-vivo* models.[48] The alarmin HMGB-1 that stimulates inflammation through the RAGE receptor is an important factor in generating scars[49] and has also been shown to be highly upregulated by culturing BMSCs in hypoxic conditions.[50] Whether HMGB-1 is released in the secretome of BMSCs is not known. However, some data support the notion given that, in bone marrow cells cultured in hypoxic conditions, HMGB-1 is released.[51] Other factors, such as fatty acid exposure, can also induce a proinflammatory phenotype in BMSCs.[52] When comparing BMSCs to ADSCs, Sukho et al[53] showed that conditioned media from ADSCs induced a more anti-inflammatory M2 state than did the conditioned media from BMSCs. Therefore, when considering BMSCs for therapeutic development, the secretome of BMSCs cultured in hypoxic conditions might be more proinflammatory than that from BMSCs cultured in normoxic conditions and, regardless of the culture conditions, the conditioned media from ADSCs promotes a noninflammatory M2 state better than does the conditioned media from BMSCs. In comparison with BMSCs, hypoxic culture conditions for the ADSCs had little effect on a cell's phenotype or the contents of its secretome.[54] BMSCs become activated and home in on the inflamed tissue through inflammatory cytokines that prime MSCs for chemotaxis.[55] During the inflamed state with blood infiltration, invading BMSCs will express high levels of the Wnt family member 5A (WNT5A) protein,[56] shown to be associated with cancer development and progression,[57] and release proangiogenic and immunosuppressive factors that increase the immunosuppressive and angiogenic capacities of tumors, thus rendering the cancer cells more aggressive and more prone to metastasis.[58] BMSC transplants are associated with an increased risk for cancer,[59] including melanoma.[60] Conditioned media from BMSCs, acting through a cytokine network,[61] has been shown to upregulate antiapoptotic and proliferative genes in cocultured breast tumor cells that correlate with tumor progression and poor prognosis[62] and also promote angiogensis in prostate and breast cancer cells[63] as well as in colorectal cancer.[64] *In-vivo* studies have shown that the conditioned media from human BMSCs induced gastric cancer growth,[65] while other studies revealed that the *in-vivo* effects of induced cancer growth can be mediated by the exosomes of BMSCs.[66] One mechanism by which BMSCs might induce cancer is through the conversion of the normal fibroblast phenotype to the CAF phenotype by the BMSC secretome.[28] BMSCs also secrete chemokine (C-C motif) ligand 5 (CCL5) protein, observed to mediate BMSC actions in promoting breast cancer metastasis[67] and to mediate the proliferation of prostate cancer cells by the BMSC secretome.[68] BMSCs have also been demonstrated to promote breast cancer metastasis through the activation of neutrophils. Tumor necrosis factor alpha (TNF-α) is part of the inflammatory response and is released by macrophages and other cells. TNF-α-activated BMSCs expressed high levels of CXCR2 ligand, which activates a G-protein-coupled receptor and triggers a variety of intracellular signaling cascades that mediate numerous pathological processes in cancer.[69] When coinjected with mammary tumor cells in mouse mammary glands, TNF-α–activated BMSCs recruited more neutrophils to the tumor. In turn, the recruited neutrophils dramatically promoted the invasion and migration of tumors cells and promoted their metastasis in lung colonization.[70] The conditional medium of human BMSCs promoted the proliferation, migration, and invasion of PC-3 prostate cancer cells, and the expressions of MMP-2 and MMP-9 in PC-3 were upregulated.[71] Vallabhaneni et al[72] demonstrated that the microRNA and protein composition of secretome from BMSCs is tumor-supportive, and more than 30 percent of proteins are antiapoptotic and cell-proliferative. In another study, BMSCs induced platelet activation, and the platelets then enhanced the effects of BMSCs on tumor proliferation and metastasis,[73] suggesting that the use of PRP and the secretome of BMSCs in conjunction with one another might have very procancerous effects. In contradistinction to the procancerous effects of BMSCs in both *in-vivo* and *in-vitro* models, ADSCs suppressed cancer growth *in vivo*.[74]

Chen et al[75] showed that BMSCs secreted differential levels of numerous cytokines compared to dermal fibroblasts, such as significantly greater amounts of epidermal growth factor, keratinocyte growth factor, insulin-like growth factor 1 (IGF-1), vascular endothelial growth factor α, platelet-derived growth factor with two B subunits, erythropoietin, and thrombopoietin but significantly lower amounts of IL-6 and osteoprotegerin. Although IGF-1 plays a role in wound healing, the expression of IGF-1 in BMSCs is extremely high—49-fold higher at the messenger RNA (mRNA) level and 22-fold higher at the protein level in the conditioned medium compared to dermal fibroblasts—and might pose health risks when the exposure is high and constant. For example, many studies have shown that IGF-1 is a primary mediator of the effects of growth hormone (GH) and increases the probability of cancer,[76] including breast cancer.[77,78] The exosomes released from BMSCs also contain mRNA that can promote a state of dormancy in breast cancer cells,[79] which is a state that can protect cancer cells from chemotherapy for years.[80] BMSCs also recruit macrophages, part of the inflammatory response that further induces proliferation of cells.[81] Interestingly, when the skin is wounded where macrophages and other immune cells infiltrate the skin and interact with epithelial stem cells (EpSCs), the EpSCs present an epigenetic memory of the wounding event that elicits a more proliferative phenotype.[82] Given that BMSC secretome recruits macrophages,[75] the BMSCs might be contributing to the epigenetic memory in EpSCs that induces the

more proliferative phenotype. Macrophage recruitment also means that more IL-4, secreted by a number of immune cells, including T-cells and macrophages,[83,84] will be present in the tissue, with a resulting propensity for the development of scleroderma.[85]

Further, BMSCs seem to induce proliferation, migration, and chemotaxis,[86] as well as differentiation of resident stem cells to somatic cells[87] by releasing factors such as GDF11.[88] Not only will frequent cycling of stem cells lead to depletion (exhaustion) of local stem cell populations,[89] but we can also think of cancer as wounds that do not heal,[90] with the wounds characteristic of constant cell cycling. Wounds have long been known to promote cancer,[91] and the recruitment of BMSCs,[81] normally not present in skin in significant numbers, during wounding might thus play a role in developing the cancerous phenotype through the aforementioned mechanisms involving IGF-1 and macrophages. BMSC transplants are known to be associated with recurring cancer,[59,92] whereas ADSC transplants have not been associated with cancer,[93,94] and ADSC exosomes have even been shown to suppress cancer growth.[95] Like most cell types, BMSCs exist as a number of phenotypes, with one major phenotype resulting in collagen deposition, expression of proinflammatory mediators, and a reversal of the MSC-established suppressive mechanisms of T-cell activation. The mechanism of fibrosis are similar in all tissues, and one phenotype of BMSCs is fibrogenic, expressing glioma-associated oncogene homolog 1 identified in the human and mouse bone marrow, where it plays an important role in bone marrow fibrosis.[96] That BMSCs induce a rapid inflammatory and healing response for initial wound closure and infection control is illustrated by recent results where the administration of BMSCs to patients with lower-extremity chronic wounds only works to reduce the wounds for the initial two weeks of treatment, whereas, at 12 weeks, there are no effects.[97] For complete wound healing beyond the initial M1, profibrotic, and proinflammatory responses induced by BMSCs, the secretome of ADSCs is required to promote the M2 anti-inflammatory phase of wound healing.[53,98] Furthermore, transplantation of BMSCs leads to an aging effect in the tissue as measured by the p16 biomarker.[99] How this is related to the exhaustion of the endogenous stem cell pool and whether the secretome from BMSCs also induces the increased p16 aging effect is not known. BMSC transplants have been reported in several studies to induce toxic epidermal necrolysis,[100,101] an extreme inflammatory response. Toxic epidermal necrolysis (TEN) involves inflammatory natural killer (NK) cells,[102] and the mechanism by which BMSCs induce TEN might include the enhancement of interferon-γ (IFN-γ) release. In this regard, coculture of BMSCs and NK cells, even without cell-cell contact and therefore acting through the BMSC secretome, was shown to enhance IFN-γ production in NK cells.[103]

Unlike ADSCs, experimental data have provided evidence that some fibroblasts in fibroblast foci (fibroblast clusters in fibrotic tissue) can be derived from BMSCs. The circulating peripheral blood-derived fibroblasts derived from BMSCs (called "fibrocytes") have fibroblast-like properties and express CD45+ collagen I+ CXCR4+.[104] BMSC-derived fibrocytes can be chemotactically gathered to damaged lung tissue sites and play a key role in the establishment of fibrosis at the injured sites.[105]

We must also consider that tumor cells and mesenchymal stem cells migrate from the primary tumor site to the bone marrow,[106] and therefore, using BMSCs and their secretome for therapeutic development or for skin care products (AnteAGE®; Irvine, California) might be compromised by a cancer phenotype, as well as the likelihood of inducing inflammation, overproliferation, fibrosis, and replicative stress.[88]

ADSCS

In a seminal study, ADSCs and their exosomes derived from cancer patients are safe and of therapeutic benefit, indicating that expanded ADSCs donated by cancer patients remain unaffected by patient condition, including cancer.[107] This is in contrast with BMSCs, which present a number of potential dangers to the patient.[108] Although ADSCs serve as a precursor for many cell types, their most important function is signaling to surrounding cells, inducing the cells to differentiate into specialized cells, including dermal fibroblasts and keratinocytes.[109] Signaling from ADSCs in the skin is so important that it is even necessary for the activation of epidermal stem cells,[110] including those in the hair follicle.[111] ADSCs from the dermis are able to differentiate into multiple cells types, including adipocytes[112] that are required for wound healing.[113] Adipocytes themselves, differentiated from ADSCs, have been successfully used to treat soft-tissue diseases when injected subcutaneously. In a study, treated patients were followed for one year without adverse events.[114] In an animal model of diabetic ulcer, nanofat-containing ADSCs, implantation was used to successfully close the wounds[115] and treat facial wrinkles in human patients.[116] Interestingly, under identical culture conditions, BMSCs are more inclined than ADSCs are to differentiate into adipocytes.[117] Dermal adipocytes are necessary for maintenance and healing of the skin given that they secrete a number of cytokines and growth factors and recruit fibroblasts to wounds.[113] Skin-derived adipose mesenchymal stem cells (SMSCs) mediate potent anti-inflammatory effects in the surrounding tissue, including the immunomodulation of macrophages into an anti-inflammatory phenotype,[118] exhibiting M2 anti-inflammatory characteristics.[119] SMSCs also inhibited the induction of pathogenic T-helper type 1 (Th1) and Th17 cells and markedly suppressed the development of experimental allergic encephalomyelitis in a mouse model of multiple sclerosis.[120] SMSCs have also been shown to be superior to BMSCs in healing spinal cord injuries in an *in vivo* rat model.[121] ADSCs have been shown to heal skin,[122] including the chronically irradiated skin of cancer patients,[123] pressure ulcers, even if derived from diabetic patients,[124] acid burns,[125] atopic dermatitis,[126] and many other organs, including the lungs and liver,[127] and have additionally been found to inhibit B-cell proliferation, thereby shifting the cytokine profile of B-cells toward an anti-inflammatory profile.[98] In diabetic patients, the wound healing of ulcers might be augmented with the inhibition of advanced glycation end-products (AGE) given that Gong et al[128] showed that AGE inhibited the proliferation of ADSCs, leading to human ADSC apoptosis, and inhibited endothelial cell-directed differentiation. In a rabbit model of skin wounds, ADSCs exhibited better epithelial regeneration and collagen deposition into the wound and better forming of normal, nonscarring skin tissue with normal architecture compared to BMSCs.[129] The wound healing effects of the secretome from ADSCs appears to be mediated, in part, by the large fraction of molecules in the secretome that are

devoted to ECM regulation and remodeling,[130] reepithelialization, collagen deposition, and neovascularization.[131] Although hypoxic culture conditions for the ADSCs have little overall effect on the secretome,[54] a number of proteins involved in ECM regulation and scar-free healing are upregulated, including collagen III.[130] Part of the wound healing effects of ADSCs might be mediated by their paracrine actions by enhancing the proliferation of fibroblasts and keratinocytes.[132] In chronic wounds, the healing phases are all prolonged, with significant infiltration by neutrophil granulocytes.[133] The secretome of ADSCs can interrupt this cycle and allow wound healing to occur by decreasing the inflammatory state, promoting cell migration and proliferation, as well as by the induction of angiogenesis and by controlling fibroblast migration and proliferation. This has been demonstrated in digital wounds treated with occlusive dressings that maintain a moist wound environment with preservation of the endogenous ADSC and fibroblast secretome within the wound site.[134] ADSC secrete macrophage migration inhibitory factor, one of the proteins that has been identified to inhibit fibrosis.[135] In liver fibrosis, similar to in skin, an overproduction of collagen by fibroblasts during the damaged state leads to scar tissue. The therapeutic effect of ADSCs on the symptoms of cirrhosis and liver fibrosis is based on ADSC-secreted growth factors, anti-inflammatory effects on stellate cells (a type of pericyte), and the antifibrotic and angiogenic effects of ADSC-secreted proteins.[136] In a murine model, ADSCs prevented fibrosis in steatohepatitis by suppressing IL-17-mediated inflammation.[137] The ADSC secretome has been used to reduce the scarring (involving fibrosis) following acne lesions in an animal model.[138] The secretome from ADSCs is also critical to the development and maintenance of hair follicles.[111] In humans treated with fractional carbon dioxide laser, the recovery phase was augmented with the conditioned media from ADSCs, where erythema, melanin, and transepidermal water loss were reduced, while mRNA for procollagen III was enhanced 2.6 times compared to the control.[139]

Punch grafts from human patients containing ADSCs have been used to successfully treat chronic ulcer wounds in a randomized, controlled study.[140] ADSCs also can switch activated-M1-like inflammatory macrophages to a M2-like phenotype,[141] an anti-inflammatory phenotype[142–145] able to reduce adipose tissue inflammation and reduce insulin resistance.[146] The ability of the secretome from ADSCs to drive the M2 anti-inflammatory state is superior to that of the secretome from BMSCs.[53] The ADSC secretome has also been shown to decrease the lipopolysaccharide-induced increases in M1 marker expression by inhibiting both the mitogen-activated protein kinase and nuclear factor κ B pathways, and increased the expression of M2 markers by activating the signal transducer and activator of the transcription 3 pathway.[147] AMSCs, acting through the molecules they release, also reduce MMP-1 expression in ultraviolet-irradiated human dermal fibroblasts,[148] have antioxidant effects,[149] reduce the histological and immunochemical consequences of photoaging,[150] and significantly reduce ultraviolet-induced skin wrinkles.[109] ADSCs also regulate dermal fibroblasts to secrete more collagen type III,[151] known to be involved in scarless instead of fibrotic wound healing as mediated by the exosomes released by ADSCs.[21] Antiaging effects of ADSCs in the skin have also been observed through the reduction of AGE levels[110] and the regulation of melanocyte proliferation, melanin content, tyrosinase activity, and tyrosinase mRNA levels through low levels of IL-6 in the conditioned media.[152]

Several proteins released more abundantly or exclusively by ADSCs—namely, AXL, CCL2, CLU, CRLF1, LGMN, and PCSK9[153]—were found to be significantly associated with the regulation of neuronal death and apoptosis. These bioactive factors might contribute to the modulation of neuroinflammation exerted by the secretome of ADSCs in preclinical models of neuropathic pain by activating different mechanisms. AXL is a receptor tyrosine kinase that regulates the innate immune system activation and which controls the phagocytosis of dead neurons. Furthermore, in a model of neurodegeneration, Maguire et al[154] showed that the secretome from four cell types, including ADSCs and FBs, was able to rescue neurons and other cells from oxidative stress that would have otherwise killed the cells.

The results of ADSCs are sometimes confused with those from whole-fat tissue. While whole-fat-tissue transfer has been successfully used to heal digital ulcers in patients with systemic sclerosis,[155] oncological follow-up for up to 10 years following fat transfer has suggested no increased risk for cancer to the patient.[156] A meta-analysis of 1,453 patients with a mean follow-up period of 16.3 months (range: 1–156 months) in fat grafts for breast reconstruction showed no increased incidence of breast cancer.[157] *In-vitro* and *in-vivo* studies have indicated that AMSCs inhibit cancer growth,[92] as would be expected given that they are known to build the ECM, and a normal ECM is important to inhibiting cancer growth through a process of dynamic reciprocity.[158] In human cells,[159] *in-vitro* studies show that fat tissue but not fat-derived stem cells increases breast cancer xenograft growth rates significantly. *In-vitro*, three-dimensional culture studies of ADSCs further suggest their safety in breast tissue, as the ADSCs formed acinar-like structures and showed characteristics of epithelial differentiation when stimulated by the breast epithelial cell line HBL-100.[160] The safety profile in ADSCs is relatively well characterized, including nuclear stability as they proliferate,[161] making these cells a valuable tool for cancer therapeutics,[162] including the delivery of paclitaxel to cancer patients.[163] A one-year follow up of patients with an average age of about 50 years who were receiving injections of autologous ADSCs for osteoarthritis observed no adverse events together with improved pain levels and diminished disease progression.[164] In a Phase II clinical trial, patients were followed for six months following the injection of allogeneic ADSCs for the treatment of perianal fistulas. No related adverse events were observed and closure of the fistula was realized in most patients.[165] In another Phase II clinical trial, patients were followed for two years following the injection of allogenic ADSCs for Chron's fistula without adverse side effects and with complete healing observed in 80 percent of the patients.[166] In a Phase IIb double-blinded, randomized, placebo-controlled study of ADSCs injected for osteoarthritis, significant improvement and no adverse events were seen in a six-month follow-up.[167] Thus, the safety profile of ADSCs and their secretome, even when injected, is well established in humans.

Indeed, using *in-vitro* and *in-vivo* methods, Lee et al[168] showed that the secretome from ADSC attenuates the proliferation and migration of B16 melanoma cells in culture and in a mouse xenograft model, suggesting that the secretome of ADSC might be useful for anticancer therapeutic development. Also, one study

indicated that ADSCs inhibit the proliferation and induce apoptosis of hepatic cancer cells,[169] while Ko et al[94] demonstrated that the exosome fraction of the secretome derived from AMSCs suppressed hepatocellular carcinoma growth. In comparison, the secretome from BMSCs promotes the metastasis of hepatocellular carcinoma growths.[170] Xie et al[171] showed that the conditioned media from ADSCs was able to suppress liver cancer cell growth through the downregulation of epithelial mesenchymal transition signaling, and Yu et al[172] found that the conditioned media from ADSCs reduced cancer cell viability by an induction of cell apoptosis and S-phase-arrest in bladder tumor cells. In a melanoma mouse model, the conditioned media of ADSCs was shown to have an antiproliferative effect on melanoma cells and shown to occur through cell-cycle arrest and apoptosis of tumor cells, and ADSCs themselves were suggested to have an inhibitory effect on the growth of A375SM cell-derived tumors *in vivo*.[173] In a breast cancer model, Ryu et al[174] demonstrated that ADSCs inhibit proliferation and induce apoptosis of MCF-7 breast cancer cells. ADSCs have been revealed to have an inhibitory effect on the proliferation of androgen-responsive (LNCaP) and androgen-nonresponsive (PC3) human prostate cancer cells.[175] Another possible mechanism through which the secretome of ADSCs can suppress cancer is through the inhibition of the mammalian target of rapamycin,[176] a powerful activator of proliferation of cells that is upregulated in cancer.[177]

ADSCs have also been shown to modulate immune function in a number of favorable ways, reflected in the ability of the secretome from ADSCs to enhance the survival of skin allografts.[178] A number of studies have revealed that ADSCs were able to inhibit activated T-cell proliferation with or without direct ADSC-T cell contact,[179] and the secretome alone from ADSCs can suppress T-cell proliferation, differentiation, and activation.[180,181] Coculture of peripheral blood mononuclear cells with ADSCs resulted in the inhibition of proinflammatory T-cells and the induction of T-cells with a regulatory phenotype, characteristic of an anti-inflammatory response.[182] Also, in a 32-month follow up of a human case study where ADSCs were derived from liposuction of the patient, bone reconstruction was demonstrated without any inflammation for the duration of the 32-month study.[183] Autologous ADSCs were also used for erectile dysfunction in a Phase I, three-month follow up with significant results and no adverse events.[184] The lack of expression of MHC-II proteins in ADSCs that have been shown to be present in the secreted exosomes of BMSCs is another reason for the successful allogeneic administration of the ADSC secretome and not the BMSC secretome.[185] In a murine model of phorbol-12-myristate-13-acetate-induced dermatitis, administration of mouse ADSCs at the site of dermatitis significantly dampened the neutrophil oxidative burst, protecting the tissue from damage by releasing antioxidants.[186] Similarly, using exosomes from ADSCs, motoneurons were protected from oxidative stress.[187]

Tissue sources for the collection of ADSCs might be important to the ADSC phenotype, where the tissue depot, skin versus abdomen, and health status of the donor can impart some variation to the ADSCs and their secretome. For example, ADSCs sourced from humans with obesity compared to those from normal-weight individuals secrete a more proinflammatory cytokine profile.[188] Peng et al[189] showed that ADSCs directly interact with dendritic cells, inducing a dendritic cell phenotype that allows immune tolerance and inhibits the polarization of naïve T-cells into proinflammatory Th1 cells. Furthermore, Ivanova-Todorova et al[190] discovered that ADSCs are more potent suppressors of dendritic cell differentiation than BMSCs. Ribeiro et al[191] compared the ability of ADSCs and BMSCs to suppress peripheral blood B-, T- and NK cells. Their results showed that ADSCs had a stronger inhibitory effect than did BMSCs, yielding a greater proportion of T-cells in the nonactivated state. Regulation of the T-cell phenotype in the skin is important beyond just consideration of inflammation because, for example, Ali et al[192] found that Treg cells are involved in stem cell activation in the hair follicle, making them important for hair growth and wound healing.[193] Rybalko et al[194] showed that dual injections of macrophages and ADSCs can be used as an intervention in the treatment of critical ischemia. Local injection of both cell types leads to accelerated recovery of muscle function and histopathology, reduced inflammation, and improved perfusion of the regenerating ischemic limb. They also presented evidence that ADSCs are powerful modulators of macrophage functional status as evidenced by the upregulation of CD206 and gene profiling, useful as an endogenous or exogenous anti-inflammatory macrophage phenotype for regenerative medicine.[195] Given the ability of ADSCs to drive the M2 macrophage phenotype, shown to downregulate proinflammatory stimulation by releasing anti-inflammatory cytokines and antagonizing M1 macrophage responses in wounds,[196] ADSCs are considered to be proresolving in their actions during an inflammatory state.[197] The proresolving mechanisms of ADSCs include inducing M2 macrophages to secrete growth factors that activate epithelial cells and facilitate fibroblast differentiation into myofibroblasts.[198] M2 macrophages also promote extracellular matrix turnover, clear apoptotic/necrotic cells, debris, and tissue-damaging extracellular matrix components and orchestrate TH2- and regulatory T-cell migration to the wound side,[199] thus controlling, for example, autoimmunity. Systemic administration of ADSC-conditioned media significantly attenuated lipopolysaccharide-induced bone loss by reducing serum levels of proinflammatory cytokines. The beneficial effect of ADSC-conditioned media was partly mediated by regulating the function of macrophages during inflammatory processes.[200] ADSCs can also fight infection by the release of antimicrobial peptides (AMPs), such as cathelicidin, that control chronic bacterial infections.[201] ADSCs have been shown to provide superior antibacterial effectiveness than do BMSCs, an effect found to be at least partially mediated by the LL-37 AMP.[202] AMPs are an effective and safe means to fight bacterial, viral, and fungal infections because they are not toxic to the host's cells[203] and can block proinflammatory macrophage responses.[204]

Another important quality of ADSCs is their robustness as they age compared to BMSCs. ADSC cultures retained their normal diploid (2n) karyotype better than did BMSCs, up to passage 20 for human BMSCs and passage 30 for human ADSCs.[205] Further, as MSCs double in culture, ADSCs are less inclined to express the senescent phenotype than BMSCs.[117] Subcutaneous native adipose tissue was not affected by the donor's age in terms of cellular senescence and yield of ADSCs from donors. Also, a constant mRNA level of osteocalcin and alkaline phosphatase with a similar level of matrix mineralization of ADSCs remained unaffected by donor age after

osteogenic differentiation. The secretome of ADSCs was similarly unaffected by age when used to promote angiogenesis.[206] Further, measuring the senescent state in BMSCs versus ADSCs from the same donors, Wu et al[207] showed that ADSCs were less senescent, an aged phenotype, and were better able to restore cardiac function and induce angiogenesis than matched BMSCs from older patients were able to. However, in the laboratory setting, cultured ADSCs that have reached replicative senescence and have been preconditioned with IL-2 might have been induced into a phenotype not warranted for therapeutic use.[208] In a within-subjects comparison of human ADSCs, there was no reduction in tridifferentiation viability when measured at ages 12 years apart.[209] In contrast, BMSCs lose viability as they age,[210] with, for example, a significantly reduced ability to repair ischemic tissue as they age.[211] Likewise, cryopreserved BMSCs have more limited expansion capability and are more senescent than are cryopreserved ADSCs.[212] The exosomes contained in the secretome of BMSCs have significant age-dependent differences in their immune profiles, including more proinflammatory microRNAs.[213] Thus, although phenotypic variation, including age of the stem cells, is important for therapeutic development in all stem cells, BMSCs are more negatively affected by age, doubling number, and other factors than are ADSCs.

We must also remember that the bone marrow is a site of great stem cell homing, both from the bone marrow to other regions of the body and from other parts of the body back to the bone marrow, including cancerous stem cells that return to the bone marrow and engraft there.[214] Not only can BMSCs and their secreted molecules induce cancer,[215] including melanoma,[60] but they can also transfer cancer from the donor to the recipient,[216] including by the exosomes of the BMSCs.[217] This happens despite careful screening of BMSCs, because cancerous BMSCs and other cancer stem cells recirculate to the bone marrow and engraft there,[214] remaining dormant and undetected for years.[218] Therefore, migration of adult stem cells, including cancerous phenotypes, occurs not only to and from the bone marrow, but also within the bone marrow.[219] Thus, if BMSCs are being used to generate cytokines for the development of skin care products, the use of cytokines from cancerous BMSCs is a possibility. Moreover, because stem cells from many parts of the body home to bone marrow and engraft there,[220] stem cells carrying any sort of injury signal incurred in that part of the body suffering injury, where a state of repurposing of fetal development genetic programs might be switched on in adult stems cells,[221] might be present in the bone marrow. Such cells in the bone marrow might possess the proliferative, cancer-like phenotype,[82] potentially leading to cancer in the body as the BMSCs are recruited to fight infection and regenerate tissue at sites of injury.[222] Malignant cells in the bone marrow alter the phenotype of surrounding stromal cells to express key niche factors such as CXCL12 and Jagged1 that, in turn, promote malignant hematopoietic cell growth.[223] The cytokines released from the BMSC cancer phenotype might contribute to the pathogenesis of cancer, as demonstrated in chronic myelomonocytic leukemia patients[224] and acute myeloid leukemia,[225] through altered cytokine secretion.

Furthermore, because T-cells from various areas of the body migrate to the bone marrow, cytotoxic CD8+ T-cell phenotypes generated during infection can induce BMSCs to release a proinflammatory set of cytokines, including IL-6.[226] Thus, using the cytokines from BMSCs in a skin care product might include proinflammatory cytokines that have been sourced from BMSCs stimulated by cytotoxic CD8+ T cells, therefore inducing inflammation when applied to the skin. IL-6 is associated with chronic inflammatory states and is a direct regulator of breast cancer stem cell self-renewal,[227] additionally promoting metastasis of hepatocellular carcinoma.[170]

CONCLUSIONS

Considering the many molecular components of the secretome and the actions of those many molecules within defined biochemical pathways in a number of target cells in the skin, the secretome from ADSCs and fibroblasts provide a safe and efficacious means for therapeutic development. In contrast, significant health problems can result from using blood-derived cells and their secretome, namely platelets and BMSCs. Therefore, although the secretome from BMSCs is efficacious in driving a classic M1 inflammatory response and cellular proliferation, the BMSCs and their secretome might only have a place in the development of products for short-term use in treating the initial healing phase of wounds with severe infection. Given the many risks posed in the use of BMSCs, including for the approved treatment of blood diseases,[108] and that ADSCs have been shown as safe[107] and effective in ameliorating some of the adverse events associated with bone marrow transplants for blood diseases[228] and given the potential of ADSCs to differentiate into blood cells[229] or aid in the differentiation of BMSCs into blood cells,[230] ADSCS might one day even supplant the transplant of BMSCs for leukemia and other blood diseases, in addition to their optimal use in developing skin care products and in performing dermatological procedures.

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