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Review

Adipose-derived cellular and cell-derived regenerative therapies in dermatology and aesthetic rejuvenation

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ABSTRACT

Cellular and cell-derived components of adipose-derived tissue for the purposes of dermatologic and aesthetic rejuvenation applications have become increasingly studied and integrated into clinical practice. These components include micro-fragmented fat (nanofat), the stromal vascular fraction (SVF), adipose-derived mesenchymal stem cells (ASC), and extracellular vesicles (EVs), which have all shown capability to repair, regenerate, and rejuvenate surrounding tissue. Various aesthetic applications including hair growth, scar reduction, skin ischemia-reperfusion recovery, and facial rejuvenation are reviewed. In particular, results from preclinical and clinical studies are discussed, with a focus on clarification of nomenclature.

1. Introduction

This review summarizes the use of adipose-derived products in hair growth, scar improvement, skin ischemia-reperfusion (I/R) recovery, and facial rejuvenation. A brief historic overview is provided, as well as discussion on adipose collection techniques and regulatory guidelines.

1.1. History

Fat grafting has been utilized for over 100 years, with the first fat graft described by Van der Meulen in 1889 for the treatment of diaphragmatic hernias (Bellini et al., 2017). In this case, attempt at fat placement between the liver and diaphragm was unsuccessful due to the large volume of fat resorption. The first successful fat graft was reported by Neuber in 1893 for the correction of facial scarring (Neuber, 1893). Although the results seemed initially favorable, the long term outcome was less optimal due to inadequate survival of the transplanted fat. An additional limitation was difficulty quantifying the amount of viable transferred fat, which varied depending on the resulting damage to cells during the harvesting and processing stage. To improve survival outcome, a systematic procedure was developed by Sydney Coleman aimed at improving the survival of cells by careful handling of the fat during harvesting, purification, and grafting (Coleman, 1995, 1998). The Coleman technique is still widely used today in current plastic surgery practice. Further studies done by Zuk et al. in 2001 and 2002 showed that lipoaspirate contains a mesenchymal stem cell (MSC) population comparable to that isolated from the bone marrow, thereby expanding opportunities in multiple fields (Zuk et al., 2002, 2001).

1.2. Adipose collection techniques

Cellular and cell-derived biological products can be autologous or allogeneic, meaning these products are manufactured from either the patients' own cells or obtained from cells not belonging to the patient, respectively. The manufacturing process typically involves three steps: adipose harvesting, processing, and implantation. Fat harvesting involves the aspiration of subcutaneous fat through use of a cannula. Historically, liposuction was performed under general anesthesia without the use of vasoconstricting agents, thereby resulting in significant blood loss with potential need for blood transfusions. Jeffrey A. Klein introduced the tumescent analgesia technique in 1985

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(Venkataram, 2008), whereby the subcutaneous layer is infiltrated with large volumes of crystalloid fluid containing a local anesthetic agent (e.g. lidocaine) with a vasoconstrictor (e.g. epinephrine) prior to aspiration, thereby eliminating the need for general anesthesia and limiting blood loss (Klein, 1993; Venkataram, 2008).

1.3. Regulatory guidelines

In the United States, human cells, tissues and tissue products (HCT/Ps) fall under the United States Code of Federal Regulations (CFR) and are under the regulatory purview of the Food and Drug Administration (FDA) as established by the interstate commerce clause of the Public Health Service (PHS) Act of 1938. 21 CFR 1271 specifically refers to general provisions of HCT/Ps. More recently, the FDA has finalized guidance documents for physicians, surgeons, and manufacturers of HCT/Ps pertaining to the use of autologous adipose-derived products. Two guidance documents from the FDA dealing with minimal manipulation and homologous use as well as the same surgical procedure exception allow for the use of a patient's autologous fat harvested during the same surgical procedure so long as it is for homologous use and not manipulated or altered in its characteristics in anyway other than rinsing, sizing, and shaping (Sipp, 2018). Additionally, no products capable of changing the relevant characteristics of fat can be added in order to preserve the original tissue as a fat transfer or fat graft. Products harvested and utilized according to the regulations do not require either FDA pre-market approval (PMA) or a biologics license application (BLA). The PMA process evaluates the safety and efficacy of the product, while the BLA process requests the introduction of the product into interstate commerce. However, any other manipulation (in excess of rinsing, sizing, shaping); addition of medications; combination with other cellular products, or use of the fat tissue outside of the same surgical procedure (prolonged storage or transfer to another location) conversely requires the manufacturer to submit an investigational new drug (IND) application to the FDA (Sipp, 2018). This regulatory pathway is most notable as it hence applies to the practice of treating harvested adipose tissue with a tissue collagenase, which enzymatically separates the adipose tissue from its local stem cells or other nucleated cells. Such practices are considered greater than minimal manipulation, as it changes the relevant characteristics of the harvested fat, and therefore requires pre-market authorization and licensing from the FDA before it can be used in patients.

2. Products

Adipose-derived cell sources for regenerative therapy are rapidly showing promise in dermatology and aesthetic rejuvenation for several reasons, including the ease of harvesting and readily available sources in the human body.

Adipose tissue consists of components including adipocytes, fibroblasts, endothelial cells, MSCs, and hematopoietic cells. The following section provides a description of autologous components that are typically used for aesthetic and dermal applications. These components can be divided into cellular and cell-derived products depending on whether the cells or the acellular secretomes are utilized for regenerative purposes.

2.1. Nanofat

Nanofat is defined by a graft size of 400–600 μm or less (millifat 2.4 mm or less; microfat 1.2 mm or less) (Cohen et al., 2017). Mature adipocytes are fragile and easily resorbed by the body while smaller immature adipocytes have increased viability and therapeutic efficacy (Bi et al., 2018). Nanofat is easily obtained through steps of mechanical digestion and filtration of fat. However, the viability is markedly reduced due to the structural disruption of adipocytes during the isolation process (Tonnard et al., 2013). Recently, Bi et al. presented a method of emulsification using enzymatic digestion in addition to filtration of the harvested product through a 0.6 mm strainer, thereby increasing the viability and reducing tissue size (10 μm–70 μm) while preserving MSCs (Bi et al., 2018). Furthermore, a newer mechanical isolation method without addition of enzymes is gaining interest due to the simplicity and effectiveness of producing micro-fragmented adipose tissue (Tremolada et al., 2016).

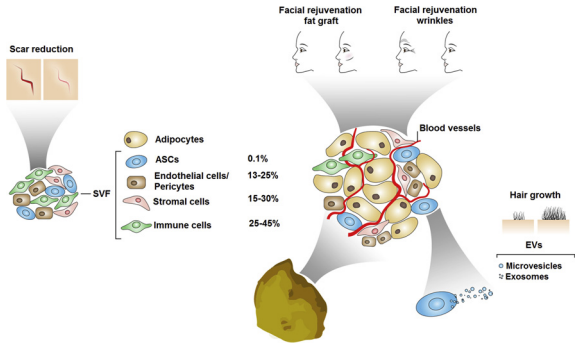

Fig. 1. Schematic of various components of adipose tissue and associated regenerative applications. Values are based on (Bourin et al., 2013). EVs, extracellular vesicles; ASCs, adipose-derived mesenchymal stem cells; SVF, stromal vascular fraction.

2.2. Stromal vascular fraction (SVF)

The SVF, isolated from adipose tissue, is devoid of adipocytes but contains various cells, including MSCs, endothelial cells, pericytes, stromal cells, and immune cells (Shah et al., 2013; Silva et al., 2017; SundarRaj et al., 2015) as shown in Fig. 1. The isolation process is performed via mechanical/enzymatic digestion, centrifugation, and filtration. The SVF is a desirable source material for cell-based therapy due to real-time isolation of adequate cell populations without culture expansion (Zhou et al., 2017).

2.3. MSCs

Unlike SVF, which is a heterogeneous mixture of cells, MSCs are a more homogenous population of cells derived from SVF. MSCs can be obtained by mechanical disruption or enzymatic digestion of fat followed by culture expansion *in vitro,* these cells are able to differentiate into several embryonic lineages, most notably the mesodermal lineage (Caplan, 1991; da Silva Meirelles et al., 2006; Dominici et al., 2006; Uccelli et al., 2008). This differentiation capacity is assessed based on the accumulation of various cell products (hydrophobic for fat, mineralized for bone, and polyanionic for cartilage) in response to specific growth factors (Dominici et al., 2006). However, this differentiation capacity, which occurs in an artificial setting, has not been explicitly shown *in vivo* (Bianco et al., 2013). Certain studies have claimed that ASCs differentiate into other cell types such as bone (Choi et al., 2014), hepatocyte-like (Yin et al., 2015), and insulin-secreting cells (Fazili et al., 2016). However, this evidence is not always conclusive or differentiation is dependent on the use of scaffolds consisting of specific materials. For example, human ASCs injected into a mouse model were claimed to differentiate into hepatocyte-like cells based on the detection of human albumin in the liver after 30 days (Yin et al., 2015). In another study, ASCs seeded onto hydroxyapatite scaffolds were transplanted *in vivo*, and were shown to differentiate into osteocytes and bone tissue within the scaffold micropores (Choi et al., 2014). Despite the controversy regarding the multipotent nature of MSCs, this product has demonstrated immunomodulatory and regenerative potential in several studies (Ezquer et al., 2009; Krause et al., 2007; Lee et al., 2006). In addition to adipose-derived MSCs (ASCs), MSCs can also be derived from several other tissues. ASCs are very similar to bone

marrow-derived MSCs (BM-MSCs) in terms of proliferation and differentiation capacity (Frese et al., 2016; Goncalves et al., 2017; Zuk et al., 2002). Studies found that ASCs have stronger immunomodulatory effects than BM-MSCs (Ivanova-Todorova et al., 2009; Melief et al., 2013; Montespan et al., 2014; Najar et al., 2010, 2013; Ribeiro et al., 2013), although additional studies are required to identify MSC sources that are optimal for specific indications. It should be noted that the MSC terminology is currently debated, as this term has been widely used for various products without specifically taking into account tissue source, degree of cell potency, and gene expression (Sipp et al., 2018). The various nomenclatures include bone marrow stromal cells, multipotent stromal cells, mesodermal stem cells, and mesenchymal stromal cells (Caplan, 2017). An emerging alternative name is medicinal signaling cells, which has been proposed as multipotency is not the primary function of these cells in the body and therapeutically injected MSC rarely differentiate. However, the overwhelming amount of MSC-based clinical trials and publications would present difficulties in adopting a new terminology.

2.4. Extracellular vesicles (EVs)

EVs are biological nanoparticles contained by a lipid bilayer without the potential to replicate (Théry et al., 2018). These nanoparticles are released by all cells, including MSCs, and can be isolated from cell culture media or patient-derived samples (Théry et al., 2018). It is well documented that EVs play an integral role in cell communication (Borrelli et al., 2018). Specifically, the regenerative potential of EVs is mediated through activation of signaling cascades that modulate growth factor expression in neighboring cells in response to injury (Montermini et al., 2015; Todorova et al., 2017). As a result, EV-based therapeutics has been gaining interest in many areas of medicine including immunomodulation, regeneration, drug delivery, and cancer therapy (Busatto et al., 2019; Lener et al., 2015; Wolfram and Ferrari, 2019).

2.5. Product characterization

Cellular and cell-derived components of adipose tissue are distinguished and quantified by specific markers (Keshtkar et al., 2018). MSCs can be characterized by the presence of CD105 (endoglin), CD 73 (ecto 5′ nucleotidase), and CD90 (Thy-1), as well as the absence of CD45 (leukocyte marker), CD34 (hematopoietic progenitor and endothelial cell marker), CD14 or CD11b (macrophage markers), CD79α or CD19 (B cell markers), and human leukocyte antigen-DR isotype (HLA-DR) (Dominici et al., 2006; Frese et al., 2016; Zhou et al., 2017). HLA-DR is a human lymphocyte antigen that is not normally expressed on MSCs, unless stimulated with interferon-gamma (IFN-γ) (Dominici et al., 2006; Goncalves et al., 2017; Teixeira et al., 2013). It should be noted that other markers may be used to further characterize MSCs; those listed above are a recommended minimal criteria (Table 1) (Dominici et al., 2006). In addition to the markers presented in Table 1, more recent literature suggests that the following markers can also be used for ASCs: CD44 +, CD29 +, CD13 +, CD166 +, CD10 +, CD49e +, CD59 +, CD31-, CD56-, and CD146- (Dubey et al., 2018; Mildmay-White and Khan, 2017).

Table 1
Minimal Criteria for Mesenchymal Stem Cell (MSC) Characterization.

Surface marker characterization	Cell Surface Markers	Presence in MSCs
Endoglin	CD105	+
Ecto-5'-nucleotidase	CD73	+
Thy-1	CD90	+
Leukocytes	CD45	–
Endothelial cell and hematopoietic progenitor marker	CD34	–
Macrophages	CD11b or CD14	–
B cells	CD19 or CD79α	–
Human lymphocyte antigen	HLA-DR	–

MSCs are also defined through two other criteria: adherence to plastic in standard cell culture conditions, and multipotent differentiation capacity. Multipotent differentiation capacity must be verified via differentiation of MSCs to osteoblasts, adipocytes, and chondroblasts (Dominici et al., 2006).

Nanofat is characterized by the presence of MSC colonies expressing the aforementioned markers as well as adipocytes and other cells associated with adipose tissue (Bi et al., 2018). SVF is characterized by a lack of adipocytes and the presence of MSCs and other stromal elements of adipose tissue. Unlike MSC characterization, SVF includes endothelial cells and hematopoietic progenitors (CD 31 and CD34) and leukocytes (CD45) among other cell types (Bourin et al., 2013; Silva et al., 2017).

Several factors influence EV characteristics, including cellular source and isolation method (Busatto et al., 2018). EVs exhibit protein markers, including tetraspanins (CD63, CD81, and CD9) and heat-shock proteins (HSP60, HSP70, and HSP90) (Keshtkar et al., 2018; Lener et al., 2015). It should be noted that there are currently no established universal protein markers for classification of EVs as a whole or EV subgroups. RNAs could potentially serve as EV-enriched markers in the future, however, due to varying reports on the specificity of RNA incorporation into EVs more studies are necessary before nucleic acids can be used for classification (Théry et al., 2018). Aside from protein markers, additional factors and properties are used to characterize EVs; however, nomenclature misuse has resulted in confusion. The terms exosome and microvesicle refer to biogenesis pathways by which EVs are formed. Exosomes are formed through the endocytic pathway, while microvesicle formation occurs upon cell membrane budding. Currently, there are no reliable markers to distinguish between these types of EVs, and the terms exosome and microvesicle should only be used if microscopy techniques confirm biogenesis (Bebelman et al., 2018; Théry et al., 2018).

3. Preclinical and clinical applications

3.1. Hair growth

The hair cycle is complex with interactions between growth factors and various signaling pathways. The hair follicle goes through phases of growth, regression, and quiescence. It is suspected that adipocytes secrete factors to promote activation of hair follicles. Specifically, the wingless/integrated (Wnt) pathway plays an important role in signaling dermal papilla cells to release factors resulting in MSC migration toward the dermal papilla, where the cells proliferate and differentiate during the growth (anagen) phase (Bernard, 2017; Jiang et al., 2017; Schmidt and Horsley, 2012). The growth factors implicated in the anagen phase include fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), insulin like growth factor (IGF), and platelet derived growth factor (PDGF) (Danilenko et al., 1996; Lin et al., 2015; Peus and Pittelkow, 1996; Yano et al., 2001). Successful studies with BM-MSC-derived products for hair growth further support the need to explore the use of alternative MSC sources including adipose-derived products. Specifically, Rajendran et al. demonstrated that BM-MSC EVs were able to promote hair growth in *in vitro* studies on dermal papilla cells and *in vivo* studies in mice. The study showed successful results in activating dermal papilla cells, increasing migration, and proliferation *in vitro*; as well as increasing conversion of hair follicles from the telogen to anagen phase *in vivo* (Rajendran et al., 2017).

Adipose-derived therapies have the potential to modify the follicular cycle in order to increase hair growth by releasing such growth factors. Specifically, mature adipocytes release factors to aid in the activation of the hair follicle during the anagen phase. It has also been

suggested that adipose loss and hair loss occur concomitantly (Peus and Pittelkow, 1996; Schmidt and Horsley, 2012). Due to the close relationship between adipose cells and the hair follicle, several studies have shown promising results using adipose-derived products for hair growth (Festa et al., 2011; Hausman and Martin, 1982).

Currently, the two FDA approved medications for treatment of genetic pattern alopecia (androgenetic alopecia) are finasteride and minoxidil (Ross and Shapiro, 2005; Shapiro and Kaufman, 2003). These drugs have several side effects, such as irritant dermatitis with topical minoxidil use (Rossi et al., 2012) and potential for sexual dysfunction with finasteride (Vogel, 2000). In addition to side effects and limited effectiveness in a subset of patients, the need for daily and indefinite use often leads to patient fatigue and suboptimal compliance. Furthermore, the newest innovation for hair restoration is the use of robotics for single follicular unit extraction in contrast to strip harvesting. Although, the robotic technique is less painful and invasive than conventional transplantation methods, it is only FDA approved for men (Rose, 2015; Rose and Nusbaum, 2014). Also, hair transplantation is generally not offered to patients with diffuse thinning, early signs of hair loss, or poor availability of the graft site. Therefore, new and improved treatments are necessary to avoid side effects and treat a broader population experiencing hair loss. The next sections of this review will discuss the specific use of adipose-derived cellular and cell-derived products for hair loss (Table 2).

3.1.1. Cellular products in hair growth

A study by Perez-Meza et al. evaluated treatment of male and female pattern hair loss patients with SVF-enhanced autologous fat grafts (Perez-Meza et al., 2017). Following a lipoaspiration procedure, the liquid fat was divided into two samples, which were processed to obtain a purified fat graft using a filter-based system (Puregraft) or SVF using enzymatic processing combined with centrifugation (Kerastem Celution). A mixture of the patient-derived SVF and fat graft was subcutaneously injected in the scalp. Hair growth in these patients was assessed 24 weeks post-injection by macro and global photography, and analyzed using phototrichogram analysis, a non-invasive technique that uses video-epiluminescence microscopy in combination with digital image analysis to assess hair regrowth. This technique yields values for hair counts, anagen and telogen ratio, and cumulative thickness. The study documented a mean increase of 31 hairs compared to baseline. A single patient served as his own control and received saline injections on the contralateral side of the scalp for side-by-side comparison. The results demonstrated a mean increase of 44.1 hairs/cm^2 on the treatment side compared to a mean increase of 1.33 hairs/cm^2 on the placebo side; potentially indicating that trauma from the injection site caused a minimal increase in hair growth. Additionally, a single patient received a fat graft without SVF, and this patient displayed less hair growth compared to the subjects in the combination group. Due to the limited number of control subjects, no conclusion regarding the ability of SVF-enhanced fat grafts to promote hair growth can be reached.

Similarly, Anderi et al. studied the effects of autologous-derived SVF in 20 patients, nine women and 11 men with alopecia areata (Anderi et al., 2018). The lipoaspirate samples were processed, digested with collagenase, and centrifuged to obtain SVF. Cell viability was counted manually with Trypan blue staining and characterized for surface proteins by flow cytometry. The results revealed viability was greater than 96% immediately after processing. However, the study noted a significant drop in cell viability after 4–6 h. Hair density, hair diameter, and the hair pull test were assessed prior to treatment, at three months, and six months. Prior to treatment, the mean hair diameter was 60.5 ± 1.8 μm, while intradermal injection with SVF increased this value to 62.8 ± 1.7 μm at three months and 80.8 ± 2.4 μm at six months (Fig. 2a). Likewise, hair density also increased after treatment (36% improvement) (Fig. 2b). The hair pull test, which is considered normal if the number of extracted hairs per cm^2 is between 0 and 1, showed that compared to preoperative results (4.35 ± 0.33 hair/cm2)

Table 2
Hair growth studies with adipose-derived products.

	Reference	Product	Study subjects	Results
Cellular products	(Perez-Meza et al., 2017)	One time subcutaneous injection of 1 mL/cm^2 of treatment: Stromal vascular fraction (SVF) and fat graft	9 patients (8 males, 1 female)	Statistically significant mean increase of 31 hairs/cm^2 compared to baseline.
		Fat graft alone	1 patient	Increase of 14 hairs/cm^2 compared to baseline.
		Fat graft and SVF (one side) vs. saline (other side)	1 patient serving as his own control	Increase of 44.1 hairs/cm^2 (treatment) and 1.33 hairs/cm^2 (saline) compared to baseline.
	(Anderi et al., 2018)	One time intradermal injection of a total of 5 ml of SVF	20 patients (11 males, 9 females)	Statistically significant increase in hair diameter in 19 patients after 6 months, no significant change after 3 months (60.5 μm at baseline, 62.8 μm at 3 months, and 80.8 μm at 6 months) Statistically significant increase in hair density in 18 patients after 3 and 6 months (85.1 μm at baseline, 120.8 μm at 3 months, and 121.1 μm at 6 months). Statistically significant decrease in hair pull test in 18 patients after 3 and 6 months (4.35 hairs at baseline, 0.9 hairs at 3 months, and 0.8 hairs at 6 months).
Cell-derived products	(Shin et al., 2015)	Weekly microneedle roller administration of a total of 4 ml of adipose-derived mesenchymal stem cell (ASC) conditioned media (CM) for 12 weeks	27 patients (female)	Statistically significant increase in hair density (105.4 to 122.7 hairs/cm^2) and hair thickness (57.5 μm to 64.0 μm) after12 weeks.
	(Fukuoka and Suga, 2015)	Monthly (every 3-5 weeks) intradermal injections for 6 sessions of 3-4 ml of: ASC-CM	22 patients (11 males, 11 females);	Statistically significant increase in the number of hairs (29 for males and 15.6 for females) before and after treatment.
		ASC-CM (one side) or saline (other side)	10 patients (8 males, 2 females)	Both treatment and saline injected sides displayed statistically significant increased hair growth; however, the ADSC-CM treated side showed a higher number of hairs when compared to the control side.

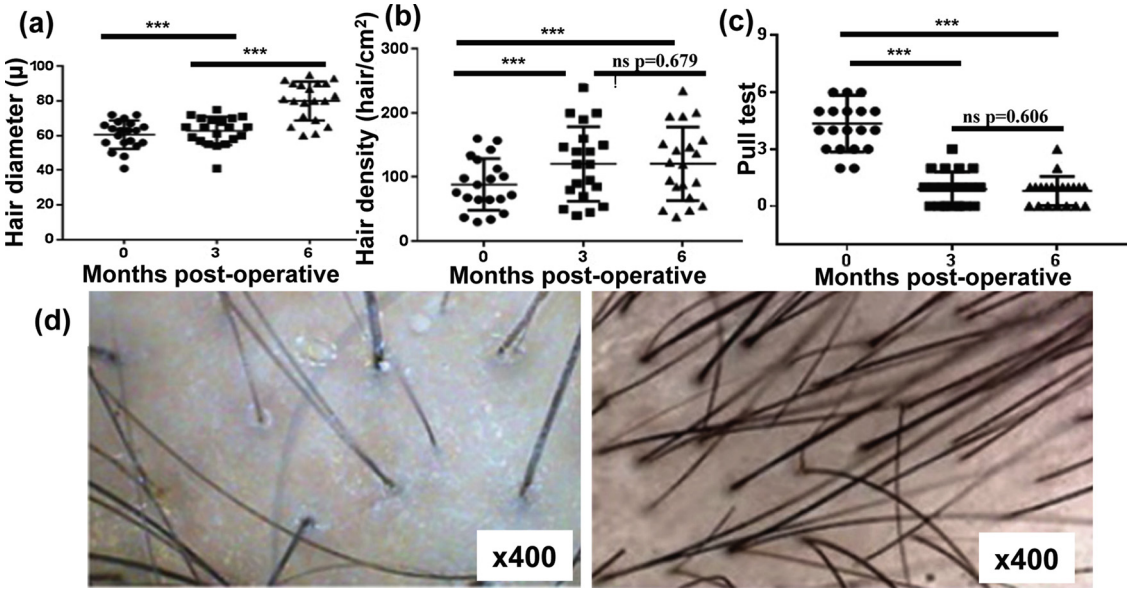

Fig. 2. Use of intradermally injected autologous SVF for clinical treatment of alopecia areata. Graphical representation of hair diameter (a) hair density (b), and pull test (c) in patients at baseline, three, and six months post-treatment with SVF. Data are expressed as mean ± standard error of the mean (SEM). Statistics by analysis of variance (ANOVA) and chi-square test. d) Photographs of hair at baseline and six months post-treatment with SVF. *, $P < 0.001$. Adapted from Anderi et al. with permission (Anderi et al., 2018). Ns, non-significant.

the number of extracted hairs at three and six months had significantly decreased to normal (0.80 ± 0.17 hair/cm^2 and 0.90 ± 0.20 hair/cm^2 respectively) (Fig. 2c). Moreover, photographs of the treated area revealed more hairs six months post-treatment compared to pre-treatment (Fig. 2d). It should be noted that the first episodes of alopecia areata resolve spontaneously within one year in most cases making the promising results from this study more difficult to interpret.

3.1.2. Cell-derived products in hair growth

The most widely studied extracellular factors in cell-conditioned media are EVs and growth factors, such as FGF, VEGF, hepatocyte growth factor (HGF), PDGF, and IGF-1. These factors have been implicated in maintaining follicular size, hair growth, and growth phases of the hair cycle (Danilenko et al., 1996; Lin et al., 2015; Peus and Pittelkow, 1996; Yano et al., 2001). A retrospective observational study by Shin et al. studied the efficacy of advanced adipose-derived stem cell protein extract (AAPE) in 27 female patients with female pattern hair loss (Shin et al., 2015). AAPE is a commercial product that contains proteins derived from ASC conditioned media (CM). The patients were administered AAPE weekly through a microneedle roller for 12 weeks (Fig. 3a). Compared to pre-treatment values, the study found a statistically significant increase in hair diameter (from 57.5 μm to 64.0 μm) and hair density (from 105.4 to 122.7 hairs/cm^2) assessed by phototrichograms (Fig. 3b–c) (Shin et al., 2015). This improvement was greater than that reported in other studies with Minoxidil (Shin et al., 2015).

Fukuoka et al. also evaluated the effects of AAPE in a study on 11 men and 11 women with androgenetic alopecia. Treatment was administered intradermally (every 3–5 weeks for six sessions) (Fukuoka and Suga, 2015) as opposed to a microneedle roller, which was used in the previously described study (Shin et al., 2015). Six of the male patients were also given finasteride in addition to AAPE during this time period. Patients were assessed with phototrichograms before treatment as well as 1–3 months post-treatment (Fig. 4a). The study found a statistically significant mean increase in the number of hairs (29 males and 15.6 females) before and after treatment (Fig. 4b). Males who had received finasteride with AAPE showed an increase in hair density, but these results were not significantly different to those males who received AAPE alone (Fig. 4c). A half-side comparison study was also done in ten patients where the left side was treated with AAPE and the right side was injected with saline (Fig. 4d). The results demonstrated that both sides displayed increased hair growth, but the AAPE was statistically superior to saline (Fukuoka and Suga, 2015).

In summary, these studies showed that both cellular and cell-derived products are safe and can lead to increased hair growth. Notably, subcutaneous, intradermal, and microneedle roller-based administration routes were all effective. Further studies are needed to elucidate the mechanisms by which these products stimulate hair follicles. Rigorously designed blinded randomized controlled trials with longitudinal tracking of outcomes are likely to provide the most accurate medical evidence of efficacy. However, the increasing amount of real world evidence, as warranted by the 21st Century Cures Act, should also be evaluated to further address applicability (Goble, 2018).

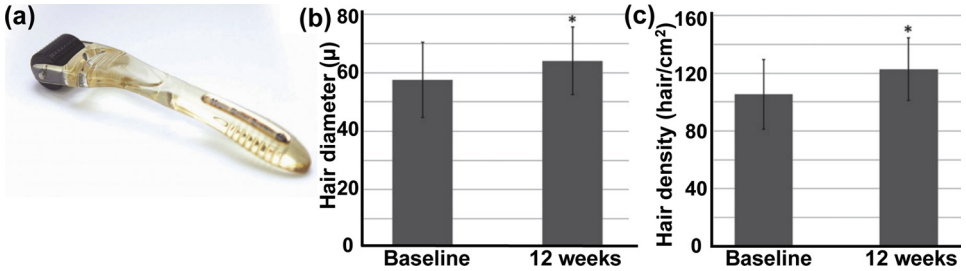

Fig. 3. Use of a micro-needle roller applied adipose-derived mesenchymal stem cell (ASC) conditioned media (CM) for clinical treatment of female pattern hair loss. a) Photograph of a micro-needle roller. Graphical representation of hair diameter (b) and hair density (c). Data are expressed as mean ± SD. Statistics by Wilcoxin signed rank test. *, $P < 0.001$. Adapted from Shin et al. with permission (Shin et al., 2015).

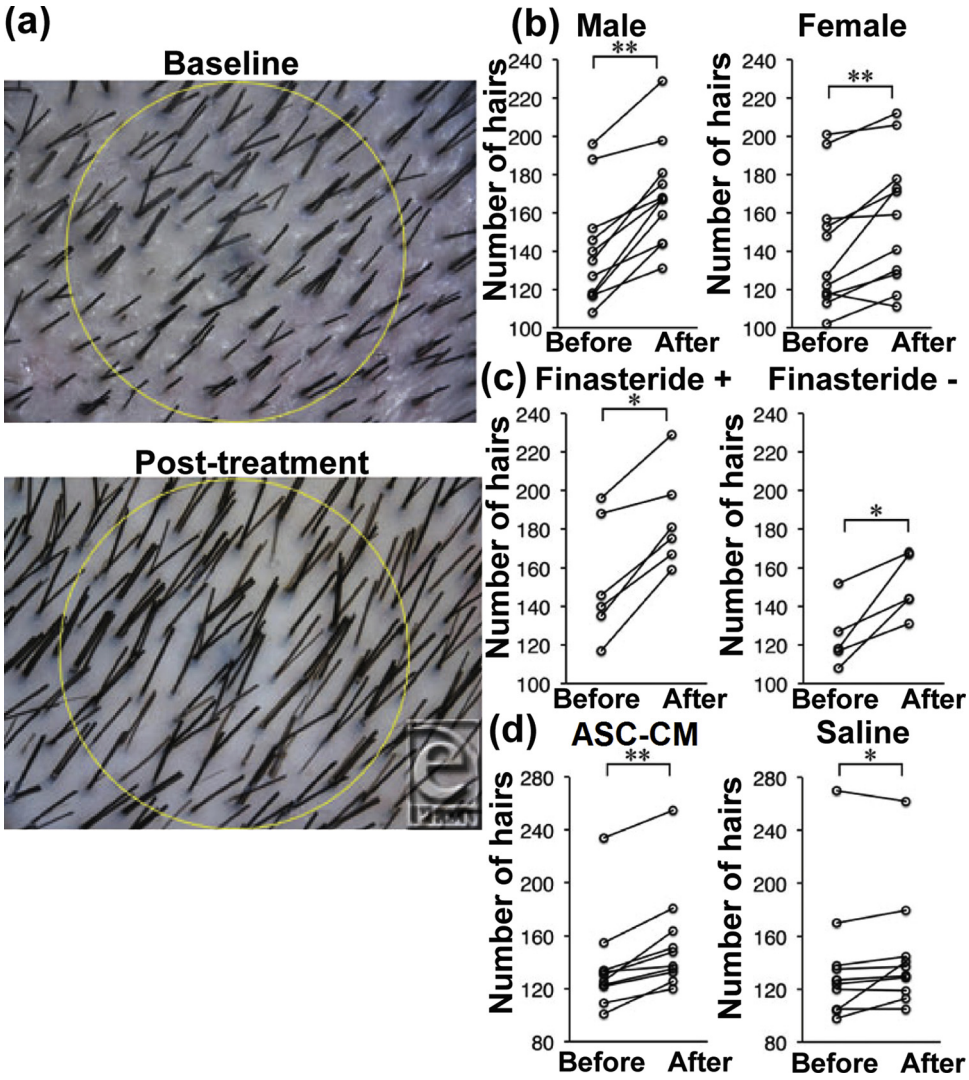

Fig. 4. Use of intradermally injected ASC-CM for clinical treatment of alopecia. a) Phototrichogram obtained images of a 24-year-old patient at baseline and seven months after treatment. Phototrichogram obtained quantitative values at baseline and after treatment with ASC-CM (b), ASC-CM and finasteride (c), or ASC-CM on one side and saline on the other side (d). Statistics by Wilcoxin signed rank test. Data are expressed as mean ± SEM. *, $P < 0.05$; **, $P < 0.01$. Adapted from Fukuoka et al. with permission (Fukuoka and Suga, 2015).

3.2. Scar reduction

Scar formation occurs in a series of steps including inflammation, proliferation, and remodeling. During these steps, there is an active role of growth factors that mediate collagen deposition induced by fibroblast stimulation and differentiation to myofibroblasts. Important regulators include a family of endopeptides called metalloproteinases (MMP), which are involved in the modulation of the extracellular matrix (ECM), and the tissue inhibitor of metalloproteinases (TIMP) (Gill and Parks, 2008; Imaizumi et al., 2009; Simon et al., 2012). Abnormal remodeling of the ECM leads to keloid formation and hypertrophic scarring (van der Veer et al., 2009; Xue and Jackson, 2015). Transforming growth factor beta (TGF-β) has been shown to be involved in the differentiation of fibroblasts to myofibroblasts (Eto et al., 2012; Montesano and Orci, 1988; Wang et al., 2017). Other growth factors, such as IGF and PDGF, have also been implicated in keloid formation (Gauglitz et al., 2011). Adipose-derived cellular and cell-derived therapies have been proposed to prevent fibrosis through anti-inflammatory and angiogenic properties (Table 3) (Kilroy et al., 2007; Rehman et al., 2004).

3.2.1. Cellular products in scar reduction

Uyulmaz et al. aimed to study the effect of nanofat on scars (40 patients), rhytids (six patients), and skin discoloration (six patients). Nanofat was prepared by emulsification and filtration steps and 1–25 mL of product was intradermally injected. Patients were assessed based on subjective satisfaction, clinical evaluations, and photographs. Results showed a softer appearance and normalization in the color of scar tissue, improvement of rhytids, and less pronounced skin discolorations. Furthermore, 92% of patients were satisfied with the results. The component(s) in the nanofat contributing to the improvement in aesthetic appearance is unclear, and further studies are necessary to separate potential ASC-mediated effects from other factors.

Yun et al. completed a pilot study using ASCs in a porcine model. Some of the required MSC surface markers were analyzed by flow cytometry but the study did not demonstrate the differentiation potential of the cells, thus not fulfilling the minimal criteria for MSC characterization. 18 full thickness incisions were made on each side of two pigs (Yun et al., 2012). 50 days after the wounds were created, one side of the pig was injected with ASCs three times every ten days into the scars, while the other side was injected with phosphate buffered saline (PBS). Differences between the experimental and control groups were significant in scar surface area reduction (11.8% in the experimental group

Table 3
Scar reductions studies with adipose-derived products.

	Reference	Product	Study subjects	Results
Cellular products	(Uyulmaz et al., 2018)	One time intradermal injections of 1-25 ml of nanofat (mean 4.6 mL)	52 patients (12 males, 40 females):	
			40 patients with scars	74% of treated scars were rated as good while 8% were rated as unchanged.
			6 patients with rhytids	66% of treated rhytids were rated as satisfactory.
			6 patients with skin discoloration	60% of treated skin discolorations were rated as good while 40% of were rated as satisfactory.
	(Yun et al., 2012)	Subcutaneous injections of 1×10^6 of ASCs (one side) or phosphate buffered saline (PBS) (other side) administered three times every ten days	2 male Yorkshire pigs	Statistically significant reduction in scar surface area in both the treatment and control groups (11.8% and 7%). Normalization of scar color was noted in both groups, with persistence of normal skin color in the treatment group with no significance between the groups. Statistically significant improvement of scar pliability in both groups with a larger improvement in the treatment group compared to the control group (41.8 vs. 37.2).
Cell-derived products	(Li et al., 2016)	Intradermal injections of 4 ml administered three times:		
		ASC-CM	3 Balb/c mice and 6 hypertrophic scar (HS) tissues	Histology of treated tissue presented more organized and thinner collagen fibers compared to the control group. Statistically significant inhibition of collagen synthesis and signaling pathway leading to significant reduction of collagen proteins and alpha-smooth muscle actin (α-SMA) in the treatment group compared to the control group.
		Unconditioned media (UM)	3 Balb/c mice and 6 hypertrophic scar (HS) tissues	
	(Wang et al., 2018)	Treatment with ASC-CM or UM administered for eight days	12 keloid explants (divided into control and treatment groups)	Statistically significant reduction of proteins related to extracellular matrix (ECM) production, inhibition of cell proliferation, and decreased angiogenic markers in the treatment group.
	(Wang et al., 2017)	One time intravenous injections of:		
		ASC-extracellular vesicles (EVs) (200 μg suspended in 200 μl PBS)	5 Balb/c mice	Gross examination demonstrated flatter scars and color more similar to surrounding skin than control group. Statistically significant reduction of scar depth, scar width, collagen density, increased ratio of collagen 3 to collagen 1 proteins in the treatment group when compared to the control groups (PBS and UM).
		EV free CM (200 μL)	5 Balb/c mice	
		PBS (200 μL)	5 Balb/c mice	
Cellular and cell-derived products	(Zhang et al., 2015)	One time intralesional injection of 0.2 ml of UM (one ear) or:	12 New Zealand albino (6 wounds created on each ear)	
		ASC (other ear)	4 New Zealand albino rabbits	Gross examination demonstrated less visible and color more similar to surrounding skin in both ASC and ASC-CM treated groups when compared to controls.
		ASC-CM (other ear)	4 New Zealand albino rabbits	Statistically significant ultrasound calculations of scar thickness in both ASC and ASC-CM treated groups compared to controls. No significant difference between untreated and UM groups.
		Untreated (other ear)	4 New Zealand albino rabbits	Statistically significant decrease in collagen proteins and α-SMA in both ASC and ASC-CM treated groups compared to controls. No significant changes between untreated and UM groups.

and 7% in the control group) and improvement in scar pliability as measured by a durometer (37.2 in the experimental group and 41.8 in the control group). Results demonstrated that the amount of mast cells in the experimental group significantly decreased ten days after the first injection, while the control group showed an increase in mast cells up to 23 days after the injection. Mast cells stimulate proliferation of fibroblasts leading to increased collagen deposition (Artuc et al., 2002; Garbuzenko et al., 2002; Rubinchik and Levi-Schaffer, 1994). Additionally, analysis of the treated scars demonstrated a decreased expression of TGF-β1 (promotes fibroblast activity (Chen and Thibeault, 2012; Clark et al., 1997)) in both groups, higher expression of TGF-β3 (inhibits TGF-β1activity (Hosokawa et al., 2003)) in the experimental group, and statistically significant increase in MMP1 (prevents excess collagen deposition (Chen and Thibeault, 2012)) was seen in the experimental group (Yun et al., 2012).

3.2.2. Cell-derived products in scar reduction

The effects of ASC-CM on hypertrophic scar *in vitro*, *ex vivo, and in vivo* were demonstrated in a study by Li et al (Li et al., 2016). The cells were analyzed for several surface markers by flow cytometry, as well as adipogenic and osteogenic differentiation potential. However, chondrogenic differentiation was not demonstrated. Six human adipose samples were acquired through lipoaspiration to prepare ASC-CM. *in vitro* studies of human hypertrophic scar-derived fibroblasts showed reduced expression of collagen type 1 (Col 1), collagen type 3 (Col 3), and alpha-smooth muscle actin (α-SMA) in response to treatment with ASC CM compared to treatment with unconditioned media (UM). The p38 mitogen activating protein kinase (p38/MAPK) signaling pathway was shown to have a role in the reduction in Col 1, Col 3, and α-SMA through suppression of p38. Similar suppression of these proteins was achieved by a p38 inhibitor (Li et al., 2016). Furthermore, *ex vivo* studies on hypertrophic scar tissues treated with ASC-CM showed a decrease in collagen proteins and α-SMA levels, compared to control groups (Fig. 5a–e). In the *in vivo* studies, mice were divided into two groups and cutaneous wounds were created prior to subcutaneous injection of ASC-CM or UM. Hematoxylin and eosin (H&E), immunohistochemistry, and trichrome staining of hypertrophic scars in the ASC-CM and p38 inhibitor groups showed less collagen deposition compared to the UM group, as well as faster wound healing at various time points (Fig. 5f–g). However, other studies have shown contrasting findings where ASC-CM promote fibrosis (Kim et al., 2009; Lee et al., 2012; Ohgo et al., 2013), which may be attributed to varying doses, growth conditions, and the pro-fibrotic state of fibroblasts prior to treatment (Kim et al., 2009; Lee et al., 2012; Ohgo et al., 2013).

In another study, the effects of ASC-CM were investigated on 12 excised keloid tissues, which were digested and cultured (Wang et al., 2018). ASCs were characterized for some surface markers by flow cytometry and the ability to differentiate into adipogenic and osteogenic lineages. However, chondrogenic differentiation was not demonstrated. Results showed a reduction in expression of plasminogen activator inhibitor-1 (PAI-1), Col 1, and TIMP-1 (Fig. 6a–c) (Wang et al., 2018), which are typically associated with the accumulation of excess ECM material leading to keloid formation (Dong and Ma, 2017; Tuan et al., 2003). The same study showed that the ASC-CM cultured keloid tissue significantly decreased the number of cells expressing angiogenesis markers (CD31 and CD34) compared to UM cultured keloid tissue (Fig. 6d–e). The reduction in proteins that regulate ECM and angiogenesis, combined with histological findings (Fig. 6f) of collagen fiber reorganization and reduction in keloid fibroblasts suggest that ASC-CM has potential to reduce keloid formation.

Furthermore, Wang et al. studied the effects of ASC-EVs on scar formation and wound healing in mice (Wang et al., 2017). Unfortunately, the study does not mention any form of MSC characterization. Moreover, the term exosome was used incorrectly as the authors did not provide any evidence of EV biogenesis. In this study, treatment with ASC-EVs led to a higher ratio of Col 3 to Col 1 mRNA in skin tissue sections compared to treatment with UM (Fig. 7a–b) (Wang et al., 2017). Immunohistochemical staining demonstrated that the ratio of TGF-β3 to TGF-β1 increased compared to UM. Quantitative analysis of scar width (Fig. 7c), scar depth (Fig. 7d), and collagen density (Fig. 7e) also demonstrated significant changes in tissues treated with ASC-EVs. Additionally, the study investigated the role of α-SMA in the differentiation of fibroblasts to myofibroblasts. ASC-EVs decreased α-SMA leading to reduced fibroblast differentiation and tissue granulation. Similar results were obtained *in vitro* in dermal fibroblasts, where mRNA analysis and Western blot demonstrated that EVs activated the extracellular signal-regulated kinases (ERK) pathway and elevated the level of MMP3, thereby increasing the ratio of MMP3 to TIMP1 (Wang et al., 2017). These results are promising as wound healing with minimal scar formation is usually associated with fewer myofibroblasts (Volk et al., 2011; Yates et al., 2012). Previous studies done by Hu et al., demonstrated that EVs promote collagen synthesis in early stages of wound healing, while inhibiting collagen deposition in later stages, which may indicate a more complex function of EVs during this process (Hu et al., 2016). In summary, both *in vitro* and *in vivo* studies demonstrated that EV treatment led to ECM reconstruction and reduced scar formation through various pathways.

3.2.3. Comparison of cellular and cell-derived products for scar reduction

Zhang et al. evaluated the effects of ASCs and ASC-CM on hypertrophic scars in a rabbit model (Zhang et al., 2015). MSCs were characterized by some surface markers as well as adipogenic and osteogenic differentiation potential. However, chondrogenic differentiation was not demonstrated. Rabbits were divided into three groups with the left ear treated with UM for all groups and the right ear treated with ASCs, ASC-CM or nothing. Photographs and ultrasounds were taken regularly to observe scar formation. The scars were harvested for further evaluation at 35 days following injection. Results showed thickening of the scars in the control sides (UM) compared to the treatment sides (ASCs and ASC-CM), which was also evident in histological analysis that showed increased contraction and thickening in the control sides (Zhang et al., 2015). Furthermore, staining showed that the ASCs and ASC-CM treated scars had an organized collagen arrangement compared to the control scars where the collagen fibers were disorderly arranged. ASCs seemed to be more effective than ASC-CM in improving various aspects of scar formation. It is important to note that side-by-side comparisons of cellular and cell-derived products can be misleading, as it is challenging to identify equivalent doses. A true side-by-side comparison would require an understanding of the amount of EVs secreted by ASCs in a specific *in vivo* environment. It is likely that both direct cell-cell contact and paracrine activation play a role in fibroblast regulation by ASC-derived products. The complexity of ECM remodeling coupled with the lack of information of specific pathways involved highlight the need for further investigation.

3.3. Skin I/R injury protection

Skin flap necrosis is a common postoperative complication following plastic and reconstructive surgery due to inadequate blood supply resulting in poor wound healing. I/R injury is caused by hypoxia (ischemia) followed by re-oxygenation (reperfusion), leading to tissue injury. Oxygen deprivation results in cell death and activation of immune cells, which release inflammatory cytokines. Regenerative therapies exploiting adipose-derived products have shown promising results in skin flap recovery by manipulating the microenvironment through growth factors and cytokines (Table 4). Furthermore, VEGF, a potent stimulator of angiogenesis, has been shown to modulate proinflammatory factors to limit I/R injury. Similarly, interleukin 6 (IL-6) has also shown to be involved in regulating angiogenic and inflammatory properties (Catar et al., 2017; Fan et al., 2008; Gopinathan et al., 2015).

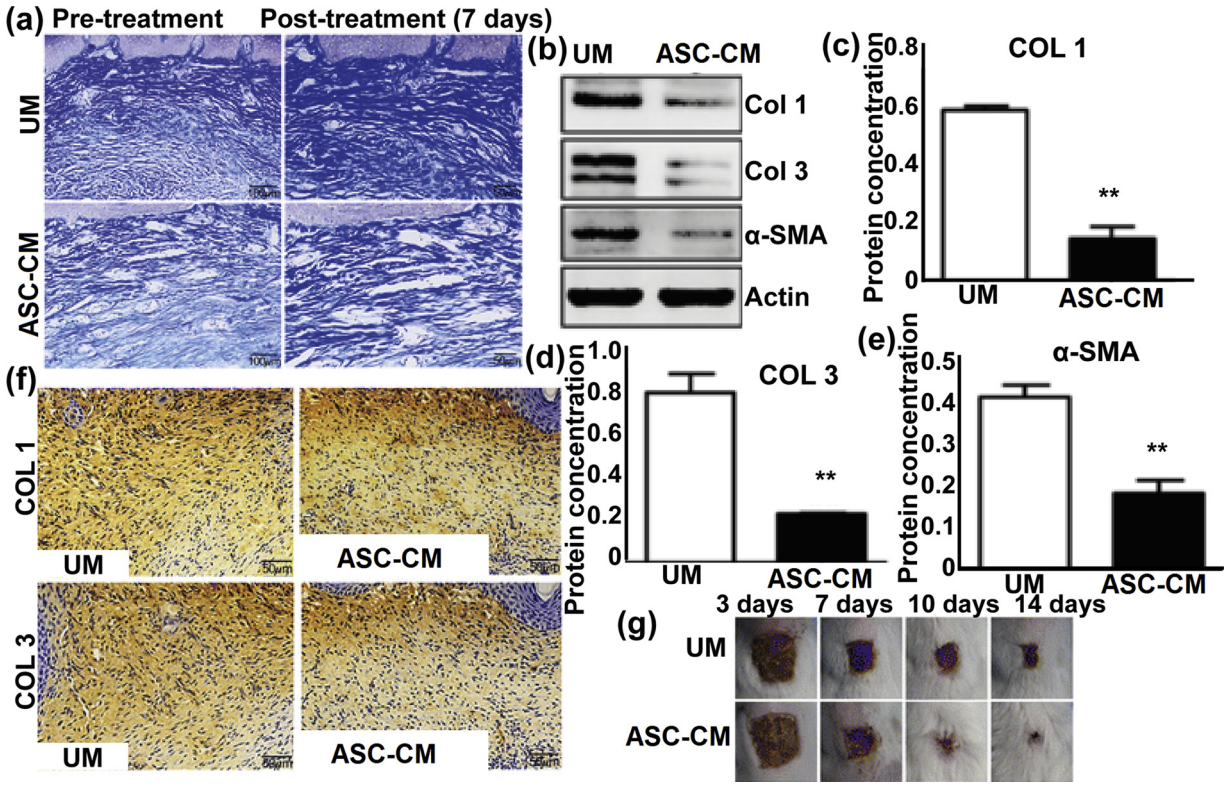

Fig. 5. Use of intradermally injected ASC-CM for treatment of scars in a mouse model. a) Masson's trichrome stained collagen fibers in hypertrophic scar tissues treated *ex vivo* with ASC-CM or unconditioned media (UM) pre-treatment (Scale bar, 100 μm) and seven days post-treatment (Scale bar, 50 μm). b) Western Blot analysis of collagen 1 (Col1), collagen 3 (Col3), and smooth muscle actin (α-SMA) in hypertrophic scar tissue after culture with ASC-CM (right) or UM (left). Histograms showing quantitative analysis of Col 1 (c), Col 3 (d), and α-SMA (e) in hypertrophic scar tissues treated *ex vivo* with ASC-CM. f) Immunohistochemistry staining of Col 1 and Col 3 in hypertrophic scars from mice treated with ASC-CM or UM. g) Global images of incisions, initially 1 × 1 cm full-thickness wound, observed at different time points after treatment of mice with ASC-CM or UM. Statistics by Student's t-test. *, $P < 0.05$; **, $P < 0.01$. Data are expressed as mean ± SEM. Adapted from Li et al. with permission (Li et al., 2016).

3.3.1. Cellular products for skin I/R injury recovery

Uysal et al. conducted an animal study to investigate the effects of ASCs in an I/R injury model (Uysal et al., 2009). Two cranial-based random flaps were elevated on the dorsum of 20 mice. ASCs were characterized for some markers by flow cytometry without evidence of differentiation capacity. One flap (control) was injected with saline and the other received saline mixed with ASCs. The flaps were then clamped for six hours to induce ischemia and subsequent clamp release resulted in tissue reperfusion. Their results indicated statistically significant increased levels of VEGF, TGF-β, and FGF in the treatment group, demonstrated through immunohistochemical staining (Uysal et al., 2009). *In vivo* differentiation of ASCs to endothelial cells was also noted, as

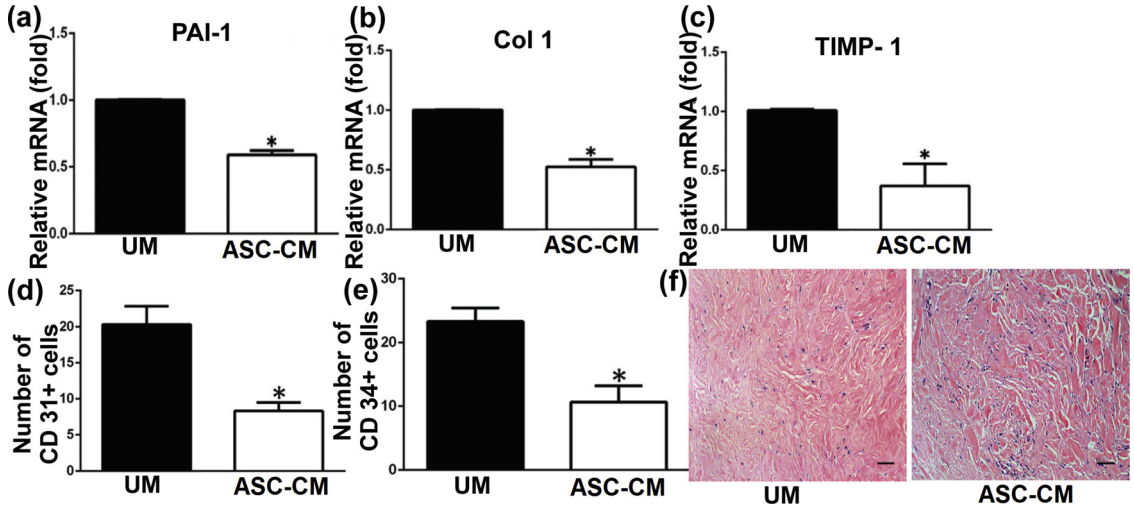

Fig. 6. Assessment of ASC-CM effects on keloid fibroblast activity *in vitro* and *ex vivo* (mouse model). a) Levels of plasminogen activator inhibitor-1 (PAI-1) (a), Col 1 (b), and tissue inhibitor of metalloproteinase 1 (TIMP1) (c) mRNA in cells treated with ASC-CM or UM. d) Amount of CD31 and e) CD34 positive cells in scar tissue treated with ASC-CM or UM. f) H&E staining of scar tissue treated with ASC-CM (right) or UM (left). Statistics by Student's t-test. Data are expressed as mean ± SD. Scale bar, 100 μm. *, $P < 0.05$. Adapted from Wang et al. with permission (Wang et al., 2018).

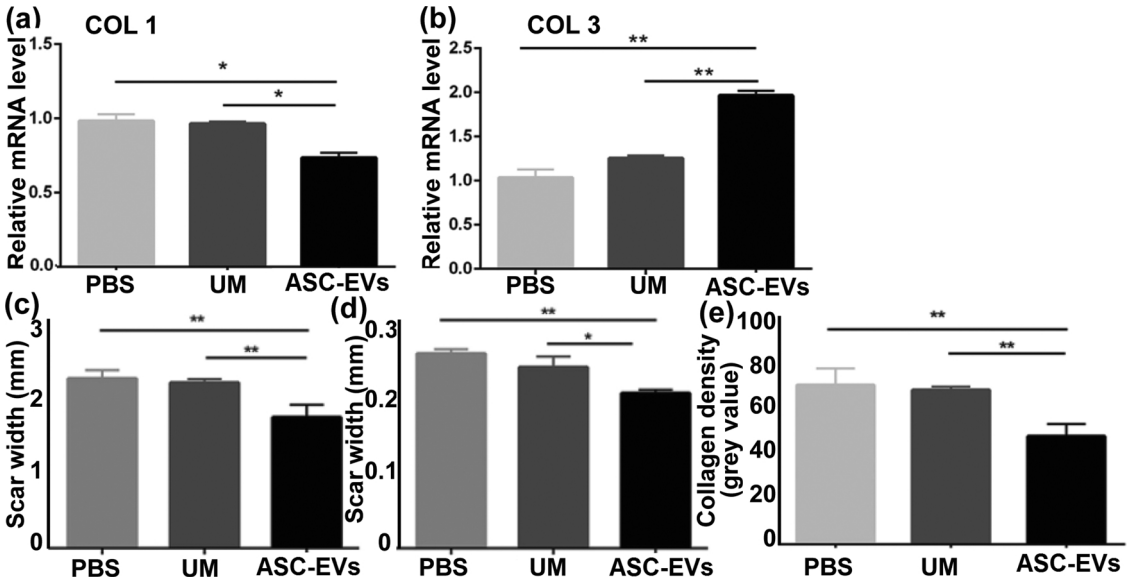

Fig. 7. Use of intravenously injected ASC-EVs for treatment of scars in a mouse model. Levels of Col 1 (a) and Col 3 (b) mRNA, scar width (c), scar depth (d), and collagen density (e) in scar tissue from mice treated with ASC-CM, UM, or phosphate buffered saline (PBS). Statistics by ANOVA. Data are expressed as mean ± SD. *, $P < 0.05$; **, $P < 0.01$. Adapted from Wang et al. with permission (Wang et al., 2017).

well as reduced tissue injury in the treatment group, as shown by scanning electron microscopy (SEM). ASCs show promising protective effects in preventing I/R injury, possibly by regulating the levels of growth factors in the microenvironment.

3.3.2. Comparison of cellular and cell-derived products for skin I/R injury recovery

Pu et al. investigated the effects of ASCs, ASC-CM, and ASC-EVs on skin flap survival in mice (Pu et al., 2017). The long thoracic vessels were ligated for three hours subjecting the pectoral skin to ischemia, which was followed by reperfusion. The flaps were then injected with ASCs, ASC-CM or ASC-EVs in the flap as well as applied between the skin and flap. ASCs were analyzed for most markers by flow cytometry and differentiation capacity of ASCs into adipocytes, osteoblasts, and chondrocytes was presented. Analysis with a 3D imaging software demonstrated that skin flap survival was enhanced by groups treated with ASCs, ASC-CM, and ASC-EVs compared to the control group. All adipose-derived treated groups also had reduced inflammation, enhanced epithelialization, more hair follicles, and higher microvessel density. Furthermore, fluorescent microscopy showed the accumulation of tagged ASCs adjacent to vessels and an increased number of cells expressing CD31. Additional studies demonstrated that ASCs expressed high levels of IL-6, which was explored as a potential therapeutic mechanism. Indeed, ASCs with IL-6 neutralizing antibodies or ASCs small interfering RNAs against IL-6 were less capable of skin repair. Although results were promising, comparisons between the three adipose-derived products were not performed, making it difficult to form any conclusions about superiority of one product over another.

In summary, ASCs, ASC-CM, and ASC-EVs were shown to enhance wound healing in skin flaps *in vivo* through modulation of growth factors and inflammatory regulators. Further studies in human subjects are necessary to draw conclusions of the clinical promise of these products.

3.4. Facial rejuvenation

Skin aging is associated with rhytids, rough texture, pigmentation, telangiectasia, skin laxity and atrophy. This process is mainly caused by a combination of intrinsic (genes, hormones) and extrinsic factors (UV, smoking) (Amirkhani et al., 2016). For instance, the imbalance of fibers within the ECM, including collagen and elastin contributes to the aging process (Schwartz and Fleischmajer, 1986). Adipose-derived cellular products, such as fat grafts, SVF, and ASCs, have shown promise in restoring collagen synthesis and decreasing elastosis (Table 5). However, high rates of product absorption require repeated procedures resulting in lower patient satisfaction. Therefore, there is a need to further improve adipose-derived products for treating facial soft tissue defects and rhytids.

3.4.1. Cellular products in facial rejuvenation

The effects of autologous SVF and ASCs were investigated in six patients undergoing face-lift surgery by examining fibers within the dermis, such as elastin, oxytalan, and collagen. Additionally, the overall architecture of the skin using orcein staining, transmission electron microscopy (TEM), and SEM was assessed (Charles-de-Sa et al., 2015). SVF-enriched fat grafts and ASCs were injected subdermally in the right and left preauricular region, respectively. MSCs were analyzed for some surface markers and differentiation potential of adipogenic and osteogenic lineages. However chondrogenic differentiation was not demonstrated. Before and after injection, portions of subcutaneous tissue from the preauricular areas were examined. Qualitative analysis showed that treatment with both SVF-enriched fat grafts and ASCs led to similar levels of new elastic fiber formation, which was demonstrated through reduced fiber diameter and rearrangements of smaller fibers in the papillary dermis, while the elastic fiber network (elastosis) decreased in the reticular dermis due to reabsorption of abnormal elastin. It is important to note that no apparent changes were visible when comparing picrosirius red staining of the connective tissue in pre-treatment and post-treatment biopsy specimens. It was suggested that potential angiogenic properties of the products enabled formation of new microvessels connecting the dermis to the subcutaneous tissue, which may mediate changes in fiber structure.

Amirkhani et al. aimed to assess the structural improvement of the nasolabial fold after autologous SVF injections (Amirkhani et al., 2016). 16 patients were recruited for this study, and lipoaspirate from each patient was collected, processed enzymatically, and characterized by flow cytometry. The final product was subcutaneously injected into the skin and nasolabial grooves. Photographs were taken prior to and six months post-injection, and evaluated using a skin scanner (Visioline) and elasticity measurement device (Cutometer) with multiprobe adapter measuring multiple variables. Treatment led to a significant

Table 4
Skin I/R injury recovery studies with adipose-derived products.

	Reference	Product	Study subjects	Results
Cellular products	(Uysal et al., 2009)	One time 1×10^7 cells in 1 mL PBS intradermal injection into the flaps	20 ICR mice Right flap (treatment) Left flap (control)	Statistically significant increase in viable area in treatment side compared to control side (21 mm^2 vs. 13 mm^2) after 7 days. Statistically significant increase in number of vessels in treatment side compared to control side (7.5 vs. 4.5). Statistically significant increase in VEGF, transforming growth factor-beta (TGF-β), fibroblast growth factor (FGF) in treatment group compared to control group.
Cellular and cell-derived products	(Pu et al., 2017)	One time intradermal injection into flap of : 1×10^6 ASCs in 120 μL of saline	6 C57BL/6J mice	Statistically significant increase in number of vessels in ASC treated group compared to the control group.
			6 Knock out (interleukin 6) IL-6 mice	Statistically significant increase in flap survival area in I/R + ASC in knock out mice compared to the control knock out mice (> 60% vs. < 20%). Statistically significant increase in number of vessels in ASC treated knock out mice compared to the control knock out mice (12 vs. 2)
		ASC-CM ASC + IgG-CM ASC + anti IL-6 antibody-CM Si-Scramble-ASC-CM Si-IL-6-ASC-CM	6 C57BL/6J mice each group	Statistically significant increase in flap survival area in ASC-CM treated group compared to groups against IL-6. Statistically significant increase in number of vessels in ASC-CM compared to groups against IL-6.
		ASC-EVs	6 C57BL/6J mice each group	Statistically significant increase in flap survival area in ASC-Evs treated group compared to the control group (> 70% vs. < 10%). Statistically significant increase in number of vessels in ASC-Evs treated group compared to the control group (14 vs. 6)

increase in dermis density and thickness, although obvious phenotypic changes in the nasolabial groove were not observed (Amirkhani et al., 2016). Furthermore, a colorimetric assay in the pigmentation and melanin production in the skin after six months remained unchanged. However, there was a significant reduction of the percentage of water evaporation from the skin surface. Notably, within a week of treatment the injected cells were not detectable, suggesting that paracrine mechanisms were primarily responsible for changes in dermis structure rather than forming a structural component of the tissue. The authors speculated that a potential mechanism for the therapeutic effects of SVF may be the replenishing of CD44, a hyaluronic acid receptor that is found in both membrane-bound and soluble forms, as this molecule has previously been found in SVF (Papakonstantinou et al., 2012). A reduction in CD44 is associated with skin aging causing the dermis layer to decrease in density (Papakonstantinou et al., 2012).

Earlier studies done by Yoshimura et al. evaluated whether SVF could improve the performance of autologous fat grafts in six patients with facial lipoatrophy due to Parry Romberg syndrome or lupus erythematous profundus (Yoshimura et al., 2008). Following a standard lipoaspiration procedure, samples were enzymatically processed to obtain SVF. Three patients received SVF-supplemented fat grafts and three received non-supplemented fat grafts. Photographs were taken pre- and post-treatment. Clinical evaluations were done prior to treatment and between nine and 13 months. Patients were classified into categories ranging from excellent to poor improvement by four blinded plastic surgeons. Results showed cosmetic improvement in all patients; however variations between the two groups were not statistically significant (Yoshimura et al., 2008).

Furthermore, Gontijo-de-Amorim et al. investigated the effects of SVF-enriched fat grafts compared to fat grafts alone in 30 patients with facial deformities following trauma, tumorectomy, or patients with Parry Romberg syndrome. The lipoaspirate was mechanically processed and the resulting SVF was characterized by flow cytometry. Photographs were taken prior and post-treatment with either SVF-enriched fat grafts or fat grafts alone. Computed tomography (CT) scans of ten patients were obtained between one and two years after the procedure to assess the amount of remaining fat. CT scans demonstrated that there was a statistically significant decrease in fat volume loss in the treatment group (9.6%) when compared to the fat graft alone group (24%). Overall, volume retention as well as subjective skin quality was better in the SVF-enriched group compared to the group with fat grafts alone. A potential explanation for the ability of SVF to improve fat grafts is the promotion of angiogenesis, which creates a more supportive skin environment (Lee et al., 2009; Rehman et al., 2004). Other studies have explored exposing ASCs to a hypoxic environment prior to injection to further increase secretion of angiogenic factors, such as VEGF (Lee et al., 2009).

In another study, Wei et al. examined the ability of PRF to improve the performance of SVF/nanofat mixture in 139 patients with soft tissue depression (Wei et al., 2017). Patients were assessed by software analysis of skin photographs and subjective satisfaction. 62 patients received a mixture of SVF, PRF, and nanofat (treatment group) while 77 patients received a mixture of SVF and nanofat without PRF (control group). After 12 months, over 90% of patients in the treatment group and less than 70% of patients in the control group were satisfied with the results. Notably, patients in the treatment group showed significant improvement of rhytids and soft tissue depressions for up to 24 months. Similar improvements were observed in the control group at six months without statistical significance after 12 months.

In summary, there are many promising opportunities for the use of adipose-derived products for facial rejuvenation, and there is a need to explore whether extracellular factors can cause similar reorganization of fibers as seen with their cellular counterparts.

Table 5
Facial rejuvenation studies with adipose-derived products.

	Reference	Product	Study subjects	Results
Cellular products	(Charles-de-Sa et al., 2015)	One time subdermal injections: SVF -enriched fat graft (1 mL) ASC (0.4 mL)	6 patients (1 male, 5 females) Right side (preauricular region) Left side (preauricular region)	Both treatments resulted in an increase of oxytalan and new elastic fibers in the papillary dermis, and a reduction of elastic fibers in the reticular dermis; however, no significant changes were visible when comparing pre- and post-treatment biopsy specimens.
	(Amirkhani et al., 2016)	One time subcutaneous injection SVF at a dose of 2×10^7 nucleated cells	16 patients (1 male, 15 females)	Nasolabial grooves showed no phenotypic changes. No changes were seen in the pigmentation and melanin production. Statistically significant decrease in water evaporation from skin, and statistically significant increase in dermal density and thickness were demonstrated.
	(Yoshimura et al., 2008)	One time subcutaneous and muscle layer injections: SVF-enriched fat graft (100 mL) Fat graft (133 mL)	3 patients (1 male, 2 females) 3 patients (1 male, 2 females)	Both treatments resulted in cosmetic improvements; however no significant differences were seen between the two groups.
	(Gontijo-de-Amorim et al., 2017)	One time subcutaneous injections of a mean volume of: SVF-enriched fat graft (58 mL) Fat graft (53 mL)	15 patients (8 males and 7 females) 15 (6 males and 9 females)	Statistically significant decrease in mean volume loss in SVF-enriched fat graft group (9.6%) as compared to the fat graft alone group (24%) in ten patients (five from each group).
	(Wei et al., 2017)	Subcutaneous injections of a mixture of: Nanofat, SVF, and platelet-rich fibrin (PRF) in varying amounts or Nanofat and SVF without PRF were administered 10.0-16.5 ml in the tempora 2.5-5.0 ml in the geisoma 10.0-25.0 ml in the frontal part 0.4-1.0 ml in the palpebra superior 0.4-1.0 ml in the palpebra inferior 0.5-2.0 ml in the lacrimal groove 2.0-4.5 ml in the zygoma (risorius) 8.0-18.5 ml in the cheeks 2.0-3.5 ml in the nasolabial groove 3.0-5.0 ml in the chin 0.8-2.0 ml in the marionette lines 4.0–6.0 ml in the submaxilla contour line	62 patients (12 males, 50 females) 77 patients	Statistically significant improvement in skin texture, elasticity pore size, and moisture in group treated with nanofat, SVF, and PRF at 12 months compared to pre-treatment assessments. Statistically significant improvement in skin texture, elasticity, pore size, and moisture in both treatment groups at six months compared to pre-treatment assessments.

4. Conclusion

Recent studies have shown that aesthetic utilization of cellular and cell-derived products is safe and relatively effective. Various adipose-derived products, including nanofat, SVF, ASCs, and EVs, have demonstrated regenerative and anti-aging potential. Although there are limitations regarding sustainable therapeutic effects, combinations of multiple adipose-derived products have shown potential in extending the viability of the administered material. Progress in this field has been hampered by the lack of standardized nomenclature for adipose-derived products, variability in assessment methods, non-evidence based claims of therapeutic efficacy, and, in many cases, circumvention of regulatory requirements.

The United States FDA regulates all cell and cell-derived products as drugs that require a BLA before products can be approved for marketing. The studies discussed in this review are first generation regenerative therapies, where products are typically transplanted within the same surgical procedure as allowed by the FDA within the practice of medicine (Sipp, 2018). Furthermore, the current regulatory framework is in place to translate findings from first generation therapies into next generation off-the-shelf or streamlined therapies. Next generation regenerative therapies would be required for greater commercial success and broader applications, necessitating appropriate preclinical trials that pave the way for phase I, II, and III trials. Moreover, as provisioned in the 21st Century Cures Act, products may be requested for regenerative medicine advanced therapy (RMAT) designation at the time of IND submission to expedite the product to market process in a more efficient manor than traditional drug research and development (Vaggelas and Seimetz, 2019).

Finally, it should be noted that the majority of studies discussed in this review did not follow the minimal criteria for defining MSCs. As a result, improved protocols and standardized procedures are needed to ensure consistency across clinical sites. Additionally, the mechanisms by which these products promote hair growth, scar reduction, and facial rejuvenation are not well understood, and require further study. Nevertheless, preliminary clinical results are paving the way to initiate more extensive clinical trials to further investigate the efficacy of adipose-derived products in the field of dermatology and aesthetic rejuvenation.

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