

Application of adipose-derived stromal cells in fat grafting: Basic science and literature review (Review)

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Abstract. Autologous fat is considered the ideal material for soft-tissue augmentation in plastic and reconstructive surgery. The primary drawback of autologous fat grafting is the high resorption rate. The isolation of mesenchymal stem cells from adipose tissue inevitably led to research focusing on the study of combined transplantation of autologous fat and adipose derived stem cells (ADSCs) and introduced the theory of 'cell-assisted lipotransfer'. Transplantation of ADSCs is a promising strategy, due to the high proliferative capacity of stem cells, their potential to induce paracrine signalling and ability to differentiate into adipocytes and vascular cells. The current study examined the literature for clinical and experimental studies on cell-assisted lipotransfer to assess the efficacy of this novel technique when compared with traditional fat grafting. A total of 30 studies were included in the present review. The current study demonstrates that cell-assisted lipotransfer has improved efficacy compared with conventional fat grafting. Despite relatively positive outcomes, further investigation is required to establish a consensus in cell-assisted lipotransfer.

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1. Introduction

Traditional fat transplantation (fat grafting) is a well-established technique and one of the standard approaches to soft-tissue reconstruction. Due to the abundance and natural properties of fat, fat grafting remains a popular procedure for augmentation and reconstruction in aesthetic and reconstructive surgery (1). However, fat tissue is intolerant to hypoxia and ischaemia, which may cause fat resorption in the recipient areas. The resulting volume reduction and long-term unpredictability are acknowledged as drawbacks of fat grafting and both have been subjects of study since lipotransfer was popularised (2,3). Previous studies have investigated the effect of fat grafting enrichment with adipose derived stem/stromal cells (ADSCs), indicating that the technique results in increased fat graft survival when compared with traditional fat grafting (4). The aim of the present study is to explain the basic science of ADSC-assisted lipotransfer and to present the experimental and clinical data regarding stem cell enhanced fat grafting (the where, how and why) outcomes published thus far by conducting a review on previous literature.

A literature-based study was conducted using the Pubmed (www.ncbi.nlm.nih.gov/pubmed) and Cochrane (www.cochranelibrary.com) database, from the introduction of the

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Abbreviations: ADSCs, adipose-derived stem cells; AFT, autologous fat transplantation; MSCs, mesenchymal stem cells; SVF, stromal vascular fraction; EPC, endothelial progenitor cells; CAL, cell assisted lipotransfer; MRI, magnetic resonance imaging; CT, computed tomography scan; SCID, severe combined immunodeficiency

Key words: adipose stromal cells, mesenchymal stem cells, cell assisted lipotransfer, fat grafting

cell-assisted lipotransfer (CAL) technique in January 2008 to November 2016. Only original studies written in English and full text articles were included. Full text articles were then cross-referenced. The following search terms were used: 'Mesenchymal stem cells' OR 'Mesenchymal stromal cells' OR 'adipose stem cells' OR 'processed lipoaspirate cells' OR 'pre-adipocytes' OR 'stromal vascular stem cells' OR 'Cell assisted fat transfer OR lipotransfer OR lipofilling'.

2. Lipofilling: Advantages and limitations

Autologous fat transplantation (AFT) has been performed since the 1890s and interest in this technique continues to increase (5). It is an advantageous reconstructive procedure commonly used to treat burns, trauma, lipodystrophies, rejuvenation and post tumour excisions (1,6). The advantage of easy and abundant harvesting of ubiquitous adipose tissue by a minimally invasive procedure explains its popularity and rapidly increasing number of clinical applications in reconstructive surgery. However, optimal outcomes are yet to be achieved, primarily due to graft reabsorption negatively impacting the procedure. The resorption rate ranges from 25 to 80% (7-11). Unpredictability and low survival rates due to partial graft necrosis are the most important drawbacks; therefore it is important to investigate how to optimize fat graft survival (2).

A number of studies have attempted to explain the lack of graft persistence and the reasons behind the poor outcomes (11,12). It has been demonstrated that inadequate vascularisation is a potential cause of fat transplant tissue loss and that revascularization of autologous fat transplants occurs after 48 h (11). This 48 h delay appears to critically impair the survival of the fat cells, which subsequently undergo degenerative changes, including cellular destruction. Following cell destruction and acute necrosis, oily cysts, calcification and connective tissue formation develop, and the fat gradually becomes absorbed (2). Studies suggest that the success of autologous fat grafting depends on the vascularity of the recipient site and early and abundant neovascularization, potentially explaining why a considerable number of mature adipocytes undergo apoptosis during the initial days of ischaemia following transplantation (2,3). The fat grafts appear to rely on the delivery of nutrients by diffusion until neovascularisation and angiogenesis are complete (11,13,14). In support of this, studies have indicated that inadequate, thicker fat grafts undergo relatively more resorption (15-17). According to Coleman *et al* (16), neoangiogenesis of the graft and consequently successful fat grafting may only be achieved when small amounts of fat tissue are placed in multiple tunnels. A previous study investigated how the quality of the graft and graft harvesting factors affect the outcome and graft survival rate (18). Mechanical disruption reduces the number of mature adipocytes to 30% and adipose tissue progenitors to 50%, indicating that any mechanical or chemical insult that damages the fragile architecture of fat tissue may contribute to partial fat necrosis (19). The congregation of ADSCs around the blood vessels may also explain why lipoaspiration alone, which spares large vessels, results in poor adipose tissue progenitors in fat grafting and poor take of the graft (20).

3. Mesenchymal stromal/stem cells

Mesenchymal stromal/stem cells (MSCs) are multi-potent adult stem cells originally identified in bone marrow (21-23). Stromal cells are characterised by their ability to differentiate into multiple lineages including adipocytes, as well as osteoblasts, chondrocytes and myocytes (24).

Since the discovery of MSCs in the 1960s their therapeutic potentials have been broadly studied. Bone marrow has been extensively studied as an MSC source for tissue engineering and regenerative medicine, and was considered for a number of years to be the major derivation source (21,25). However MSCs have been isolated from almost every tissue in the body including adipose tissue, peripheral blood, umbilical cord blood, dermis and amniotic fluid, and the field of tissue engineering is rapidly advancing (25-28).

ADSCs are a promising population of MSCs identified within adipose tissue in 2001 (24) and represent a promising alternative to bone marrow-derived MSCs for use in cell-based musculoskeletal tissue-engineering strategies (25,26). Adipose tissue is a favourable source of stem cells as it can be extracted by liposuction in large amounts with minor donor site morbidity (29). Typical isolation procedures for ADSCs involve mechanical dissociation or enzymatic collagenase digestion of the lipoaspirated tissue and subsequent centrifugation to obtain the component cellular fractions; a high density of stromal-vascular fraction (SVF) is produced. The SVF contains a highly heterogeneous cell population, including endothelial cells, endothelial progenitor cells (EPC), pericytes, preadipocytes, fibroblasts, blood-derived cells and vascular smooth muscle cells, in addition to the potential ADSCs (29). ADSCs are present as a minor fraction within the SVF and may be used directly as a source of ADSCs.

Alternatively, ADSCs may be separated from SVF and expanded *in vitro* culture by adhesion on plastic dishes (30,31). The isolation of ADSCs from the SVF is a simple, uncomplicated *in vitro* procedure. In general, cell isolation protocols include collagenase-digestion of lipoaspirate or minced adipose tissue followed by density gradient centrifugation of the enzyme-digested tissue. Stem cell yields are greater from adipose tissue than from other stem cell sources (32). As many as 1×10^7 ADSCs may routinely be isolated from 300 ml of lipoaspirate, following *ex vivo* culture, with a yield of 5,000 fibroblast colony-forming units per g of adipose tissue, which is 5x the estimate of the ADSC yield from bone marrow (33,34).

4. ADSC enhanced fat grafting: Basic science

ADSC populations have angiogenic and neovasculogenic properties, explaining why they are therapeutically attractive and advantageous to use as seeding cells for regenerative medicine (35-38). Thus, the potential for using autologous ADSCs to optimise fat transplantation has become the focus of research as the CAL technique promises long lasting results (39). CAL is a technique of fat grafting enhancement that essentially either adds *ex vivo* expanded ADSCs to aspirated fat or supplements the fat graft with the SVF cellular suspension, which is rich in progenitor cells (ADSC and EPC). This technique converts a poor ADSC lipoaspirate to a relatively rich ADSC lipoaspirate. This effect of high-level enrichment with ADSC

is considered to be due to cellular differentiation and/or paracrine signalling. *In vitro* and *in vivo* studies have demonstrated that under apoptotic and degenerative conditions, ADSCs divide, migrate and differentiate into adipocytes or vascular cells as compensation for the changes occurring during the remodelling process (40-44). However, the initial perception that engrafted ADSCs possess the ability to differentiate in order to replace injured adipocytes, endothelial cells and osteoblasts has been gradually replaced; more recent studies support that, in order to augment recovery of perfusion under ischaemia, ADSCs release soluble mediators in a paracrine fashion (45,46). It has been suggested that these mediators and angiogenic factors and not the ADSCs themselves may be primarily responsible for the trophic, regenerative and anti-inflammatory effects exhibited when ADSCs are in ischaemic hypoxic tissue (45). Through the expression of trophic factors and immune-modulatory mediators, including vascular endothelial growth factor and insulin-like growth factor-1, increased angiogenesis, capillary density and subsequently graft survival is promoted (46-52). Although both mechanisms have been supported by a number of *in vitro* and clinical studies, the science behind CAL is still under investigation and remains controversial, as it is still not clear whether this angiogenic effect promoted by engrafted ADSCs is as a result of bona fide transdifferentiation, or whether this positive effect described is arising from an alteration of the tissue microenvironment (45). The frequency with which the implanted cells undergo adipogenesis remains unclear.

Elucidating the exact fate of the implanted ADSCs and determining the optimal cell load for human use are notable topics for future research.

5. SVF vs. *ex vivo* expanded ADSCs

There are different strategies for enriching fat grafts with ADSCs (53,54). The first strategy is to isolate SVF from the fat and apply it to the recipient site. The SVF introduces stem cells and their angiogenic growth factors that subsequently promote successful vascularization, graft survival and volume retention. Another strategy is the tissue engineering approach. In this technique, the adipose derived stem cells are induced to differentiate into mature adipocytes. Growth factors are responsible for stimulating the differentiation and are therefore optimally delivered *in vivo*. Tissue engineering techniques may also utilize a scaffold system for cell delivery composed of natural or synthetic materials, or as a hydrogel. One of the few randomized controlled studies on CAL comparing conventional fat grafting to *ex vivo* expanded ADSC-enriched CAL concludes that the latter is a feasible and safe alternative that exerts a significant and substantial effect (55). However, the enzymatic isolation of ADSCs remains a major drawback as long as the *ex vivo* expansion of ADSCs is not practical for application in principalities with strict Food and Drug Administration regulations. By contrast, SVF is a mixed cell population including ADSCs, which does not require the aid of enzymatic digestion, but is disadvantaged by a markedly reduced yield of ADSCs compared with *ex vivo* culturing. Results, however indicate that the SVF technique is significantly superior to traditional fat grafting, increasing the popularity of the SVF technique in clinical practice (29,53-56).

6. Experimental and clinical trials with ADSC-assisted fat grafting

The search of publications completed for the current study yielded 15 murine (Table I) (48,52,57-69) and 19 human (Table II) (30,53-55,70-84) studies that assessed the effect of fat graft enrichment with freshly isolated ADSCs (SVF) or *ex vivo* cultured ADSCs. A total of 24 of the studies compared SVF assisted lipotransfer to traditional lipotransfer (control), 10 compared *ex vivo* expanded ADSC-enriched fat grafting to controls and one animal study compared both. Of the in-human studies, 6 were clinical trials, 7 were prospective studies, 4 were retrospective cohort studies and 2 were case reports. An overview of the retrieved studies is provided in Tables I and II. In the studies reviewed, the potential improvement of cell enriched lipofilling compared with conventional lipofilling was assessed primarily by considering two important parameters: The quality of the fat graft and the degree of fat survival, to elucidate whether CAL was superior to traditional lipotransfer; or resorption following transplantation, which is the primary disadvantage of the fat grafting technique. In the majority of studies the degree of resorption was clinically assessed whereas certain studies used less subjective imaging techniques, such as mammography, magnetic resonance imaging (MRI) and computed tomography (CT) volumetry and three-dimensional imaging. MRI volumetry is considered an exact and reproducible imaging technique (85) applied for qualitative and quantitative follow-up assessments following the grafting of autologous fat to the breast (86,87). Three-dimensional imaging is theoretically very exact but when measurements are performed in human breasts, volume deviation amounts to 60% (88). The quality of graft was assessed by the histological appearance in the animal studies and by the radiographic appearance in the human studies. The animal studies have also sought to investigate the fate of the expanded ADSC in the graft by tracking cells.

In the animal studies (n=15) cell assisted lipotransfer was compared with traditional fat grafting. Part of the lipoaspirate was used to extract the SVF, which was either used directly as a source of ADSCs or used for *ex vivo* expansion of the ADSCs. The majority of animal studies (n=11) investigated immunosuppressed mice (severe combined immunodeficiency (SCID) or nude mice) and only three investigated rabbits (59,60,69). Nude mice are the most commonly used murine model for xenografting.

The longest follow-up duration was 9 months (48). Outcomes were evaluated following extraction of the transplanted fat grafts by volume measurement and histological studies assessing fat resorption, inflammation, necrosis, vascularization and angiogenesis. Fat graft was obtained by liposuction of human fat (lipoaspiration of female donors) in seven studies, liposuction of inguinal fat in a study on rabbits and lipectomy of the inguinal fat in the other seven animal studies.

In animal studies, the evidence that enriching fat grafts with ADSCs increases volume over time is strong. All studies demonstrated a considerable reduction of fat resorption in CAL compared with fat grafting alone. Matsumoto *et al* (52) in 2006 was the first to describe the strategy of CAL, a technique that creates a stem-cell rich fat graft. The ADSC-enriched human

Table I. Murine studies.

| Study, year | Donor | Recipient | Cell type | Follow-up | (Refs.) |
|-----------------|----------|---------------------------------|-----------|----------------|---------|
| Matsumoto, 2006 | Human | SCID Mice | SVF | 4 weeks | (52) |
| Moseley, 2006 | Mice | SCID Mice | SVF/ASC | 6 months | (57) |
| Lu, 2009 | Human | SCID Mice | ADSC | 6 months | (58) |
| Zhu, 2010 | Mice | B6129SF1/J Mice | ADSC | 6 and 9 months | (48) |
| He, 2012 | Rabbits | Rabbits | SVF | 3 months | (59) |
| Piccino, 2013 | Rabbit | New Zealand Rabbits | ADSC | 3 months | (60) |
| Dong, 2013 | Human | Nude Mice | SVF | 2 weeks | (61) |
| Derby, 2013 | GFP+mice | Nude Mice | ADSC | 8 weeks | (62) |
| Fu, 2013 | GFP+mice | BALB/c Nude Mice | SVF | 56 days | (63) |
| Kakudo, 2013 | Human | BALB/cAJcl-nu/nu Mice | ADSC | 5 months | (64) |
| Zhou, 2014 | Rats | Nude Mice | SVF | 63 days | (65) |
| Garza, 2015 | Human | Nude (Crl:NU-Foxn1nu CD-1) Mice | ADSC | 7 weeks | (66) |
| Jiang, 2015 | Human | CD-1 Nude Mice | ADSC | 12 weeks | (67) |
| Paik, 2015 | Human | Crl:NU-foxn1nu Mice | SVF | 8 weeks | (68) |
| Ni, 2015 | Rabbits | Rabbits | SVF | 3 months | (69) |

SCID, severe combined immunodeficiency; SVF, stromal vascular fraction; ADSC, adipose-derived stem cells; GFP, green fluorescence protein; CD, caesarian derived; Crl, charles river laboratories; NU, nude.

Table II. Human studies.

| Study, year | N | Cell type | Follow-up time (months) | Study design | Evidence level | (Refs.) |
|--------------------|----|-----------|-------------------------|---------------------------|----------------|---------|
| Rigotti, 2007 | 20 | SVF | 30 | Case series | IV | (53) |
| Yoshimura, 2008 | 40 | SVF | <42 | Retrospective cohort | IV | (30) |
| Yoshimura, 2008 | 6 | SVF | 9-13 | Prospective cohort | III | (70) |
| Yoshimura, 2010 | 15 | SVF | <18 | Case series | IV | (71) |
| Yoshimura, 2008 | 2 | SVF | 12 | Case report | V | (72) |
| Asano, 2010 | 26 | ADSC | Unknown | Case series | IV | (54) |
| Kamakura, 2010 | 20 | SVF | 9 | Single-arm clinical trial | III | (73) |
| Tiryaki, 2011 | 29 | SVF | <36 | Case series | IV | (74) |
| Sterodimas, 2011 | 20 | SVF | <18 | Randomized clinical trial | III | (75) |
| Wang, 2012 | 18 | SVF | <6 | Case series | IV | (76) |
| Perez-Cano, 2012 | 67 | SVF | <12 | Single-arm clinical trial | III | (77) |
| Castro-Govea, 2012 | 1 | SVF | <12 | Case report | V | (78) |
| Li, 2013 | 38 | SVF | 6 | Retrospective cohort | IV | (84) |
| Peltoniemi, 2013 | 18 | SVF | <6 | Clinical trial | III | (79) |
| Tanikawa, 2013 | 14 | SVF | <12 | Randomized clinical trial | II | (80) |
| Kølle, 2013 | 20 | ADSC | 4 | Randomized clinical trial | II | (55) |
| Wang, 2015 | 12 | SVF | <6 | Case series | IV | (81) |
| Domenis, 2015 | 20 | ADSC | <12 | Prospective cohort | IV | (82) |
| Jung, 2015 | 5 | SVF | <12 | Prospective cohort | IV | (83) |

Evidence levels were used based on the hierarchical system of classifying evidence (85) as follows: I, high-quality prospective cohort study with adequate power or systematic review of these studies; II, lesser quality prospective cohort, retrospective cohort study, untreated controls from a randomised control trial, or systematic review of these studies; III, case-control study or systematic review of these studies; IV, case series; V, expert opinion; case report or clinical example; or evidence based on physiology, bench research, or 'first principles'. N, number of patients; SVF, stromal vascular fraction; ASC, adipose-derived stem cells; <, up to.

aspirated fat was transplanted subcutaneously into SCID mice resulting in a ~35% increase in average superior survival and

more prominent microvasculature genesis when compared with traditional fat grafting. However, the short follow up period

was a significant limitation of the study (52). Zhu *et al* (48) in 2010 Lu *et al* (58) in 2009 studied ADSC-assisted lipotransfer in mice 9 and 6 months post transfer, respectively. These studies demonstrated not only a higher survival rate of the fat mass compared with lipotransfer alone but proved that ADSCs contributed to vessel formation by direct incorporation into endothelial cells and the vessel wall of the neovascular capillaries, by marking the transplanted ADSCs with DiI prior to transplantation. In 2013, He *et al* (59) compared SVF enriched fat grafting to traditional fat grafting in rabbits and 3 months later the retention rate of the former had significantly improved. Also in 2013, Piccinno *et al* (60) published the results of an animal study on rabbits comparing ADSC assisted lipotransfer to traditional lipotransfer 9 months following transplantation. This was the first study where defined numbers of ADSCs were introduced into an autologous animal model to challenge the therapeutic potential of ADSCs and confirmed, once again, that ADSCs assisted fat transfer to generate a protective effect, reducing fat necrosis and increasing vasculogenesis (60). A major drawback in this study, and indeed every study investigating tissue engineering using small animal models, is the limited body fat obtainable in rabbits and the possible impact this may have on the quantity of ADSCs. Dong *et al* (61) used nude mice to demonstrate that ADSCs were the only population of SVF cells that survived the ischaemia of fat grafting, therefore the immunoregulatory function of SVF cells may be beneficial to adipose transplantation.

The 19 human clinical studies involved a total of 321 CAL procedures (30,53-55,70-84). Only seven studies yielded level II or III (high validity) evidence based on the hierarchical system of classifying evidence (85), whereas 12 (including the majority of human studies) yielded level IV or V (low validity) evidence.

The fat graft was supplemented with the cellular suspension (SVF) or ADSCs prior to transplantation. The majority of studies were on breast reconstruction and augmentation, followed by congenital, traumatic, disease related and iatrogenic (radiation-induced) soft tissue defects (30,53,54,70,71,73,75-81,83).

Patients were typically followed up for 6 to 12 months. The longest follow-up duration was 42 months (30). Outcomes were typically based on clinical examination, subjective volume estimation, imaging and patient/physician satisfaction (n=8). However, in the majority of studies more objective evaluative methods, including CT/MRI volumetry and mammography were also employed (n=11).

In 2007, Rigotti *et al* (53) presented a marked improvement in the first human study on 19 patients with radiation-induced soft tissue defects who underwent CAL. This may be considered the first example of regenerative therapies where the regenerative properties of the ADSCs were utilized to successfully treat tissue damage. A member of the same research team who first described cell-assisted lipotransfer technique (52), two years later published preliminary results when CAL had been used in two important fields of interest in reconstructive surgery: Breast augmentation and facial lipoatrophy. The results demonstrated the effectiveness of this novel technique in fat graft survival rates with favourable and uncomplicated aesthetic outcomes (30,70-72). Furthermore, in 2010 the Yoshimura group studied CAL as a safe and

efficient alternative option to implant based cosmetic breast enhancement (54). However, the team's conclusions in both studies regarding the superiority of CAL were not supported due to two serious drawbacks: Absence of a control group and formal quantitative measurements (72). More reliable evidence was published in 2011 with the first in-human prospective randomised trial comparing the SVF CAL with the traditional method in patients with facial defects (75). Follow-up assessments, six months after a single session demonstrated that patient satisfaction was significantly higher compared with non-CAL patients, although at 18 months that difference was not statistically significant. Similar satisfactory outcomes were demonstrated in breast augmentation although the resorption of SVF enriched fat graft remains unclear (81). In 2013, Li *et al* (84) favoured SVF supplementation in fat grafting over traditional fat grafting, demonstrating an enhanced clinical improvement compared with fat grafting alone (64.8 vs. 46.4%). However, Peltoniemi *et al* (79) performed the first comparative prospective study and raised doubts regarding the advantage of using stem cell enrichment in cosmetic fat transplantation to the breast. The results of this study suggested that no differences in the ADSC phenotype were observed when comparing ADSC-enriched lipoaspirate with non enriched lipoaspirate; thus suggesting that a more specific study on ADSC presence and function in the two lipoaspirates may add crucial information (79).

Despite a small number of studies exhibiting no difference, the majority of human studies clearly demonstrated a significant advantage over the non-CAL fat grafting technique; however the results were still questionable due to a lack of strong evidence. In 2013, the first two in-human randomised controlled trials were published. Tanikawa *et al* (80) performed a randomised blinded clinical trial in patients with craniofacial microsomia that compared fat grafting with and without SVF supplementation by clinical examination and CT volumetry. Although the size of the study groups was relatively small, the methodology quality was high and outcomes suggest there is a statistically significant difference in fat volume survival at 6 months (88% in CAL vs. 54% in non CAL). The second study was completed by Kølle *et al* (55) and was the second blinded randomised controlled study investigating CAL in humans. The strength of this study is that it was the first controlled clinical trial where graft specimens were histologically assessed 4 months post-transplantation. Traditional fat grafting was compared with CAL using autologous culture-expanded ADSCs. The significantly higher residual volume of 81% for the ADSCs CAL group vs. 16% in the non-CAL group was encouraging with respect to the use of this technique in tissue augmentation.

7. Optimal cell population

Of the studies included, three used different dosages of cells in order to identify the optimal cell population. Kakudo *et al* (64) in 2013 compared ADSC-enriched fat grafting in 3 different concentrations to assess traditional fat grafting and identified that 3×10^5 cells/ml is the optimal ADSC concentration.

Paik *et al* (68) in 2015 used that same xenografting murine model (nude mouse) to try and determine the optimal SVF cell concentration. Results demonstrated a dose-dependence

in the number of SVF cells that may be added to a fat graft to enhance retention by comparing fat grafts with and without SVF supplementation. Notably, this study observed that the maximum graft survival retention was a concentration of 10,000 SVF cells per 200 μ l fat.

Ni *et al* (69) performed a study that compared different mixtures of fat particles with various proportions of SVF and identified that 3:1 is the optimal fat particle-to-SVF ratio, exhibiting the greatest weight maintenance. The two latter studies suggest that there may be a minimum requirement for the amount of cells administered in order to achieve a beneficial effect.

8. Discussion

These preliminary studies suggest that ADSCs may improve the retention and volume-restoring capabilities of transplanted fat. The majority of animal studies used *ex vivo* expanded ADSC-assisted lipotransfer and demonstrated a significant improvement in graft survival and the normal histological appearance of the transplanted tissue (48,57,58,60,62,64,66,67). By contrast, in human studies, where fat grafts were typically enriched with a freshly isolated SVF containing a mixed cell population that includes a minor fraction of ADSCs, the reported results were not always substantially improved, compared with those obtained with traditional lipofilling (70-72). To the best of our knowledge, all clinical studies on CAL to date have not used a control group to objectively compare the CAL technique to conventional lipografting and in many clinical studies, the survival evaluation of the transplanted fat was based on subjective analysis of images and clinical assessment. This may introduce a potential source of observer or interpretation bias, which has the potential to affect the reliability of the results. When radiological evaluation techniques were used to allow comparison, the observer bias was eliminated by providing more objective data. A recent human study on cosmetic facial contouring, comparing traditional to SVF-enriched fat grafting demonstrated a significant increase of fat survival with SVF. The survival rate however, was only 65% for the SVF-enriched grafts vs. 46% for the control grafts (84). Therefore, based on the hypothesis that enrichment with ADSCs increases fat graft viability, the use of *ex vivo* expanded ADSC-enriched fat grafts in a similar way is expected to lead to far more reliable and predictable results than those achieved by the minor fraction of ADSCs observed in SVF and without the need for overcorrection. Undeniably, the high residual volume of >80% in a previous human randomised controlled clinical trial (68) is encouraging with respect to the potential use of enriched fat grafts in reconstructive surgery following *ex vivo* expansion of collagenase-processed ADSCs used for CAL.

Notably, to achieve these favourable results, the fat graft had to be supplemented with *ex vivo* cultured ADSCs to ~2,000x the normal physiological level (55) based on the murine studies of Lu *et al* (58) and Zhu *et al* (48), where ADSC-concentrations were increased 1,250 and 6,250x, respectively, by ADSC *ex vivo* expansion. In both studies, ADSC enrichment improved fat graft survival (Table I). However, the optimal ADSC concentration of fat grafts has not been extensively studied and a consensus on the enrichment technique is still lacking. Matsumoto *et al* (52) also identified an improvement

in graft survival using ADSCs enriched by a factor of 5, but the long-term effect remains unknown, as the duration of the study was only 4 weeks. Matsumoto *et al* (52) also demonstrated that the concentration of ADSCs in lipoaspirate is only half that in excised adipose tissue. Therefore, for comparison in cell-assisted lipotransfer, the lipoaspirate is required to be 4x the desired volume just to double physiological ADSC concentration in the fat graft. However, fat graft enrichment with ADSCs without previous expansion limits the increase to a factor of 2-5, which is potentially inadequate to exert a dynamic effect on graft survival (4). Paik *et al* (68) in 2015 demonstrated a dose dependence in the number of SVF cells that may be added to a fat graft to enhance retention and revealed that grafts receiving 10,000 SVF cells per 200 μ l of fat had the optimal volume retention. In contrast with these conclusions, Sterodimas *et al* (75) suggested that CAL provides a substantial amount of ADSCs without cell culturing. Determining the optimal cell load for human use and relating ADSC subpopulation analysis to clinical outcomes remains a challenge and requires further research.

Overall, evidence supports that ADSC augmented fat grafts are successful and may lead to superior volume survival. However, the technique has limitations: *Ex vivo* expansion of ADSCs to a high concentration is not practical for application. It also takes longer to process fat for CAL and the cost is higher when compared with traditional fat grafting. The applicability of the studies is further limited because of methods used, such as enzymatic isolation, are strictly regulated in a number of countries and principalities, including the United States (89).

Concerns have also been raised regarding the use of ADSCs in the form of CAL for breast augmentation following breast cancer therapy. Although the American Society of Plastic Surgeons has come to the conclusion that fat grafting does not increase the risk of breast cancer recurrence (90), studies have demonstrated that ADSCs may enhance the growth of proliferating cells (91-94). By contrast, no serious adverse event, including malignant transformation, has been reported following MSC treatment. However, since there remains little evidence regarding CAL therapy for breast augmentation post-mastectomy, it is currently recommended that CAL therapy should be delayed for reconstructive purposes in breast cancer treatment until the patient has been disease free for 7 years (94). Therefore producing low cost, minimally manipulated cells that may be used safely in the operating room soon after harvesting and cell isolation remains a great challenge in regenerative medical technology.

9. Conclusion

Preclinical studies and early clinical trials indicate that fat grafting enriched with SVF or *ex vivo* expanded ADSCs significantly increase residual graft volume. The beneficial effect of ADSCs on graft survival is potentially due to improved early vascularisation; promoted either by ADSC differentiation into endothelial cells or by a paracrine induced effect, although the exact mechanism of stem cell function remains unknown and requires further investigation. Although methods for tissue preparation, cell isolation and cell culture in CAL have been previously described (25,30,52,70), there remains a lack of consensus and no delineated ideal methodology for

this technique. Thus, it is critical to determine the optimal cell population (SVF or expanded *ex vivo* ADSCs) and cell load for human use in order to standardize this technique. A number of questions, including the long-term safety of CAL regarding previous cancer diagnosis and treatment, remain unanswered and long-term and larger studies are required to confirm previously documented favourable results and answer the remaining questions.

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