Thesis

Autologous Fat Grafting – Efficacy and Safety of Cell-assisted versus Conventional Techniques

A Systematic Review

submitted by

Jakob Martin Tettmann

in partial fulfillment of the requirements for the degree of

Doktor der gesamten Heilkunde

(Dr. med. univ.)

at the

Medical University of Graz

executed at the

Department of Surgery

Division of Plastic, Reconstructive and Aesthetic Surgery

under the Supervision of

Priv.-Doz. Dr.med.univ. Dr.scient.med. Hanna Luze

Univ.-Prof. Dr.med. Lars-Peter Kamolz, MSc

Graz, 21.04.2026

Statutory Declaration

“I hereby confirm that the present diploma thesis is the result of my own independent scholarly work. I also confirm that in all cases, where material from the work of others (in books, articles, essays, dissertations, and on the internet) is acknowledged, quotations and paraphrases are clearly indicated. No material other than that cited in the reference list has been used. I have read and understood the Medical University’s regulations and procedures concerning plagiarism.

Furthermore, I hereby declare that if artificial intelligence (AI) tools were used for the generation and/or correction of certain text passages in the creation of this work, such employment was conducted in compliance with ethical principles, academic integrity, and the regulations of my university. Additionally, it was ensured that this usage was transparently disclosed and appropriately attributed.”

Graz, 21.04.2026 Jakob Tettmann, m.p.

Acknowledgements

First and foremost, I would like to sincerely thank my supervisors, Univ.-Prof. Dr.med. Lars-Peter Kamolz, MSc and Priv.-Doz. Dr.med.univ. Dr.scient.med. Hanna Luze, for their valuable guidance, generous support, and patience throughout the entire process of writing this diploma thesis. I am also grateful for the opportunity to carry out this work at the Division of Plastic, Reconstructive and Aesthetic Surgery at the Medical University of Graz.

I would further like to thank my supervisors for their support in enabling the submission of the results of this work to the SFU Research Award, where it received second place in the category of wound healing.

Last but not least, I would like to thank my family, especially my parents, grandmother and girlfriend, for their continuous support throughout my medical studies, as well as my friends, who made these years especially memorable.

Abstract in German (Zusammenfassung)

Der autologe Fetttransfer (AFG) ist aufgrund seiner Biokompatibilität, guten Verfügbarkeit und vielseitigen Anwendbarkeit ein etabliertes Verfahren in der ästhetischen und rekonstruktiven Chirurgie. Eine zentrale Limitation bleibt jedoch die unvorhersehbare Resorption des transplantierten Fettgewebes, wodurch die langfristige Volumenretention erheblich variieren kann und in der Literatur Werte von unter 20% bis über 90% berichtet werden. Der Zell-unterstützte Fetttransfer (CAL), bei dem das Fetttransplantat mit Zellen der Stromal-vaskulären Fraktion (SVF) beziehungsweise adipösen Stammzellen (ADSCs/ASCs) angereichert wird, wurde entwickelt, um durch Angiogenese, Adipogenese und Geweberegeneration die Einheilung des Transplantats zu verbessern. Die klinische Evidenz im Vergleich zum konventionellen autologen Fetttransfer ist jedoch bislang uneinheitlich.

Ziel dieser systematischen Übersichtsarbeit war es zu untersuchen, ob CAL die volumetrische Retention von Fetttransplantaten im Vergleich zum konventionellen AFG verbessert und Unterschiede in den postoperativen Komplikationsraten bestehen. Hierfür wurde eine systematische Literaturrecherche gemäß PRISMA Richtlinien in den Datenbanken PubMed und Web of Science von Datenbankerstellung bis zum 30. September 2025 durchgeführt. Eingeschlossen wurden prospektive klinische Studien, die CAL mit konventionellem AFG verglichen und eine Kontrollgruppe, eine objektive volumetrische Messung, eine Nachbeobachtungszeit von mindestens 3 Monaten sowie mindestens 5 Patient:innen aufwiesen. Datenextraktion und Qualitätsbewertung erfolgten unabhängig durch zwei Reviewer. Aufgrund der Heterogenität hinsichtlich Empfängerregionen, Nachbeobachtungszeiträumen, Zellisolationsmethoden, Aufbereitungsprotokollen und volumetrischen Messverfahren wurde keine Metaanalyse durchgeführt, sondern die Ergebnisse deskriptiv zusammengefasst.

Insgesamt erfüllten 15 prospektive klinische Studien die Einschlusskriterien. 11 berichteten über eine signifikant höhere Retentionsrate zugunsten von CAL, 1 zeigte einen numerischen, jedoch nicht signifikanten Vorteil und 3 fanden keinen signifikanten Unterschied. Besonders deutlich war der Vorteil von CAL bei mehreren

Anwendungen im Gesichtsbereich, während die Ergebnisse im Brustbereich heterogener ausfielen. Klinisch relevante Komplikationen wie Ölzysten, Kalzifikationen, tastbare Veränderungen oder Fettgewebsnekrosen traten insgesamt selten auf und zeigten keine erhöhte Häufigkeit in den CAL-Gruppen gegenüber den Kontrollgruppen.

Zusammenfassend deutet die derzeit verfügbare prospektive klinische Evidenz darauf hin, dass CAL die Retention von Fetttransplantaten verbessern kann, ohne mit einer erhöhten postoperativen Komplikationsrate verbunden zu sein. Zur endgültigen Bewertung des klinischen Nutzens sind jedoch größere randomisierte Studien mit standardisierten Methoden und einheitlichen Parametern erforderlich.

Abstract

Autologous fat grafting (AFG) is an established technique in aesthetic and reconstructive surgery owing to its biocompatibility and versatility. Nevertheless, unpredictable graft resorption remains a major limitation, resulting in highly variable long-term retention rates ranging from under 20% to over 90%. Cell-assisted lipotransfer (CAL), which enriches fat grafts with cells from the stromal vascular fraction (SVF) or adipose-derived stem/stromal cells (ADSCs/ASCs), has been proposed to enhance graft survival through angiogenesis, adipogenesis, and tissue regeneration. However, clinical evidence comparing CAL with conventional AFG remains inconclusive.

The aim of this systematic review was to evaluate whether CAL improves volumetric fat graft retention compared with conventional AFG and to assess differences in postoperative complication rates. A systematic literature search was conducted in PubMed and Web of Science from database establishment until September 30th, 2025, in accordance with PRISMA guidelines. Eligible studies were prospective clinical trials comparing CAL with conventional fat grafting that included a concurrent control group, objective volumetric assessment, a minimum follow-up of 3 months, and at least 5 patients. Data extraction and quality assessment were performed independently by two reviewers. Because of heterogeneity in recipient sites, follow-up schedules, cell isolation techniques, processing protocols, and volumetric measurement methods, no meta-analysis was performed and results were synthesized descriptively.

15 prospective clinical studies fulfilled the inclusion criteria. CAL demonstrated higher volumetric take rates than conventional fat grafting in most studies. 11 of 15 studies reported significantly improved fat graft retention with CAL, 1 study showed a numerical but non-significant advantage, and 3 studies found no significant difference between techniques. The beneficial effect of CAL appeared particularly pronounced in several facial applications, whereas breast-related outcomes were more heterogeneous. Clinically relevant complications, including oil cysts, calcifications, palpable changes, and fat necrosis, were infrequent and did not occur more frequently in CAL groups compared with controls.

In conclusion, current prospective clinical evidence suggests that CAL may improve fat graft retention without increasing postoperative complication rates. Nevertheless, the available evidence is limited by small sample sizes, methodological heterogeneity, and non-standardized CAL protocols. Larger randomized controlled trials with standardized methodologies and harmonized outcome measures are required to determine the true clinical value of CAL.

Table of Contents

1 Abbreviations

3D	Three-Dimensional
ADRC	Adipose-Derived Regenerative Cell
ADSC	Adipose-Derived Stem Cell
AFG	Autologous Fat Grafting
ASC	Adipose Stem Cell
ATMPs	Advanced Therapy Medicinal Products
BAT	Brown Adipose Tissue
bFGF	Basic Fibroblast Growth Factor
BI-RADS	Breast Imaging Reporting and Data System
BMI	Body Mass Index
BRAVA	Bra-Shaped External Volume Expander
CAL	Cell-Assisted Lipotransfer
CT	Computed Tomography
EVE	External Volume Expansion
FU	Follow-Up
GRADE	Grading of Recommendations Assessment, Development and Evaluation
HGF	Hepatocyte Growth Factor
IL	Interleukin
Ki-67	Ki-67 Antigen
M	Mean
MRI	Magnetic Resonance Imaging
NA	Not Available
non-CAL	Non-Cell-Assisted Lipotransfer
PCCT	Prospective Controlled Clinical Trial
PCT	Prospective Clinical Trial
PDGF	Platelet-Derived Growth Factor
PPAR-γ	Peroxisome Proliferator-Activated Receptor Gamma
PRCCT	Prospective Randomized Controlled Clinical Trial
PRCT	Prospective Randomized Clinical Trial

PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PRP	Platelet-Rich Plasma
RCT	Randomized Controlled Trial
rpm	Revolutions Per Minute
SAT	Subcutaneous Adipose Tissue
SCID	Severe Combined Immunodeficiency
SD	Standard Deviation
SDF-1	Stromal-Derived Factor-1
SVF	Stromal Vascular Fraction
TGF-β	Transforming Growth Factor β
UBM	Ultrasound Biomicroscopy
VEGF	Vascular Endothelial Growth Factor
WAT	White Adipose Tissue

2 Figures

3 Tables

4 Introduction

4.1 Autologous Fat Grafting – Definition and Applications

There are two main types of adipose tissue: thermogenic brown adipose tissue (BAT) and energy-storing white adipose tissue (WAT) (1). While BAT is distributed throughout the cervical, supraclavicular, axillary, paravertebral, mediastinal and upper abdominal regions, WAT, the predominant type of fat in humans, is located either viscerally or subcutaneously (2–4). Subcutaneous adipose tissue (SAT) is generally present in sufficient quantities in the human body, thus rendering it an ideal autologous filling material for the correction or remodeling of profile and volume defects. In contemporary autologous fat grafting procedures, SAT is first aspirated from one area of the body and then processed before being re-injected into another area (5). In comparison with alternative filling materials, autologous fat grafting offers several advantages, including good biocompatibility, ease of availability, abundant sources, a satisfactory filling effect and minimal trauma. The broad spectrum of its applications, coupled with its established safety and efficacy record (6,7), has given rise to a significant body of research in this field, resulting in numerous enhancements since its inception (8).

4.1.1 Historical Background

The utilization of the basic principle can be traced back to as early as 1889 when Van der Meulen employed autologous fat to treat a diaphragmatic hernia (5,9). Four years later, in 1893, German surgeon Gustav Adolf Neuber successfully transplanted fat from the forearm to fill an atrophic facial scar and in 1895 Czerny performed the first breast augmentation using fat tissue on a tumor patient (10,11). However, these early satisfactory results were discredited by later reports by Strong and Peer, who observed asymmetries due to uneven resorption of the fat grafts (12,13). Subsequent years witnessed the evolution of autologous fat grafting into a clinically proven method, which was utilized in various medical specialties and in diverse anatomical regions (5,14), yet reports of varying degrees of graft failure

prevented the technique from gaining widespread acceptance until the 1980s (12,15). Coleman *et al.* were instrumental in effecting a paradigm shift in the history of autologous fat grafting (AFG) through their seminal publications. They refined and built upon the techniques established by their precursors and are widely credited with establishing the definition of AFG as a procedure consisting of harvesting, processing and reinjection of autologous fat. This constitutes a significant step forward in the direction of achieving optimum results (16–20).

4.1.2 Indications and Clinical Applications

Nowadays, AFG has become a prevalent technique in both aesthetic and reconstructive surgery, recognized as an efficacious instrument for soft-tissue augmentation (21,22). According to Luze, Strong and Suszynski, the range of applications extends to encompass a broad spectrum of anatomical sites:

- Face: facial rejuvenation, facial lipoatrophy (e.g. Parry-Romberg syndrome), scleroderma and rhinoplasty
- Breast: postmastectomy reconstruction, postaugmentation recontouring, postoperative breast deformities, congenital breast or chest defects (e.g. tuberous deformity, Poland syndrome) and micromastia
- Hand: Dupuytren's contracture, Raynaud's syndrome and hand rejuvenation
- Skin and soft tissue: pathological scar revision (e.g. burn injuries), skin rejuvenation and body contouring
- Other applications: laryngoplasty, gluteoplasty, mastoidectomy defects, etc. (12,23,24).

4.1.3 Composition of Autologous Fat Grafts

The composition of fat grafts, which are utilized in all the areas mentioned above, remains largely consistent post-harvesting. While adipocytes, otherwise known as fat cells, comprise approximately 90% of the volume, they account for only around 20% of the cells contained within the graft. In addition to these, the stromal vascular fraction (SVF) constitutes the remaining 80%. This is an amalgamation of various

cell types, including endothelial cells, pericytes, fibroblasts, macrophages, monocytes, lymphocytes, smooth muscle cells and most importantly autologous stem cells (ADSCs), which collectively form a complex structure within an extracellular matrix (25–29). Adipose tissue was previously considered to be a passive energy storage mechanism. However, recent research has demonstrated its regenerative potential, which comprises a range of processes including angiogenesis, peripheral nerve repair, enhancement of skin thickness and elasticity and the reversal of fibrosis resulting from radiation therapy, scarring, or inflammatory conditions. A substantial body of research has underscored the healing capabilities of autologous fat grafting, a phenomenon that is presumably attributable to the presence of ADSCs (26). Physiologically, adipocytes derive from these ADSCs, by either proliferation or differentiation (30). This mechanism also takes place following the injection of a fat graft into the recipient site. Due to the mechanical stress experienced during the grafting process, some adipocytes undergo apoptosis or necrosis. However, at the same time, new fat cells are created from ADSCs, helping to compensate for the volume loss caused by the death of adult adipocytes (25).

4.1.4 Theories of Graft Survival

Despite the increasing use of AFG and the wealth of research on the subject, the actual mechanism of how adipose tissue grafts survive is not fully understood (31). At present, three principal theories explaining fat graft survival following avascular surgical transplantation are widely recognized. Emerging evidence indicates that all three mechanisms may contribute concurrently to the overall graft survival process. These theories are known as the cell survival theory, the graft replacement theory and the host replacement theory (21).

4.1.4.1 Cell Survival Theory

The cell survival theory, alternatively referred to as the graft survival theory, was first described by Peer in 1955 and continues to be cited in numerous contemporary studies. It is the oldest and most significant of the three theories (31,32) and can be

stated by Peer as follows: In the context of human subjects, cells in free autogenous transplants have been observed to demonstrate a propensity for survival and the maintenance of their normal tissue structure, on the condition that they are transferred as intact cell units to appropriate transplantation sites. However, in the absence of cell survival, the transplant is replaced by connective tissue. This replacement does not correspond to the original tissue in terms of structure or function (32). For the transplanted cells to survive, it is imperative that an initial nutrient supply is furnished by plasma diffusion until neovascularization occurs at the recipient site. The latter process is predominantly promoted by perivascular ADSCs, which release angiogenic factors in response to ischemia. To ensure successful graft revascularization, each individual fat graft droplet must establish an interaction with a capillary at the recipient site (33). In consideration of the aforementioned factors, grafts with reduced volume and evenly distributed small fat droplets demonstrate an enhanced capacity for survival compared to large volume grafts (32,33). Furthermore, to ensure optimal survival of adipocytes and ADSCs, it is recommended that the employed harvesting and injection technique is as gentle as possible and water, oil and erythrocytes are removed during processing to obtain a concentrated and uniform mass. Although the cell survival theory is widely accepted, it cannot fully account for the clinical outcomes of AFG alone, suggesting the existence of other mechanisms that influence graft survival (31).

4.1.4.2 Graft Replacement Theory

According to the graft replacement theory, the transplanted adipocytes only survive partway through the process, with a significant proportion of the fat cells that persist in the long-term being derived from the transplanted ADSCs (33). Suga *et al.* posit that the demise of the initially transplanted adipocytes can be attributed to the hypoxic environment present at the recipient site (34). The findings of Yoshimura *et al.*, derived from both *in-vitro* and *in-vivo* studies, support this assertion by demonstrating that, under conditions of severe ischemia, adipocytes derived from stem cells, exhibit a heightened capacity for survival (31,34,35). Based on this fact, multiple studies have already achieved positive results by adding SVF cells or ADSCs. A detailed discussion of these outcomes will be provided subsequently in

this thesis. Nevertheless, the authors themselves recognize the necessity for further research in this area and acknowledge the variability of results (33,36,37).

4.1.4.3 Host Replacement Theory

The host cell replacement theory posits that the transplanted cells are not viable and instead undergo necrosis, being entirely replaced by host-derived cells (35). The necrotic grafted tissue is substituted with fibrous tissue, newly formed adipocytes, and neovascular structures originating from the recipient site (21). It is therefore vital to note that the condition and microenvironment of the recipient site play a critical role in determining graft survival (33). As demonstrated in a study conducted by Luze *et al*., a significant correlation was identified between the thickness of the subcutaneous fat layer at the recipient site and the take rate. This correlation was found to be approximately equal to the number of previous grafting sessions, the BMI, and the patient's bodyweight (38). To this day, various techniques have been developed to optimize the recipient site, leading to improved surgical outcomes in fat grafting procedures (33). This is merely one of numerous procedural modifications that have been instigated within the domain of fat grafting (8). Nevertheless, in order to comprehend these enhancements in greater depth, it is first necessary to examine the technique and procedure involved in fat grafting more closely.

4.2 Techniques of Conventional Fat Grafting

As previously stated, Sydney R. Coleman is widely credited with establishing the first standardized approach to contemporary fat grafting in 1994. The so-called Coleman technique consists of three distinct processes: harvesting, processing and grafting. It has been adopted and adapted by surgeons across the globe, owing to its capacity to consistently enhance fat graft survival via a methodology that is both standardized and reproducible (39). Its extensive implementation has resulted in the development of numerous methods aimed at optimizing donor site preparation, fat harvesting, post-harvest processing and grafting procedures (8). Notwithstanding

this wide array of methods, a consensus on a gold standard remains elusive to this day (40,41). This may be attributable to the presence of inconclusive results on a multitude of procedural variables, which in turn may have a detrimental effect on the quality of AFG and consequently the outcome (23). In addition, some confounding variables, such as mechanical factors, have been studied more intensely than other potential influences on the final outcome (25,27). The following presents a comprehensive overview of the current AFG technology and an analysis of the risk factors inherent to each procedural step in relation to the overall clinical result.

4.2.1 Harvesting

The manner in which adipose tissue is harvested exerts a substantial influence on the efficacy of subsequent fat graft integration (42). Despite the ongoing debate surrounding the optimal method for obtaining the most viable and functional cells for grafting, no clearly superior harvesting technique has been established to date (43). Factors such as the selection of the donor site, the administration of tumescent anesthesia, cannula size, and the utilization of power- or ultrasound-assisted liposuction can substantially influence both the initial viability of adipocytes during harvesting and the differentiation capacity of SVF cells following extraction and transplantation. It is imperative that these variables are given due consideration in order to establish a gold standard and optimize graft survival rates (24,25,42).

4.2.1.1 Harvest Site

A substantial body of research has demonstrated that autologous fat grafts harvested from various donor sites, including the abdomen, flanks, thighs and knees, show no significant differences in terms of weight, volume, or histological properties such as integrity, inflammation, fibrosis, cyst formation and neovascularization, in both preclinical and clinical investigations (42,44–46). Despite these findings, there are individual studies claiming a higher concentration of ADSCs in adipose tissue harvested from the lower abdomen and inner thighs compared to other regions (47). It has also been argued that, depending on the harvesting site, advanced donor age may exert either a negative or positive

influence on graft survival (48). However, the validity of both these hypotheses is contingent upon further validation through additional research. Li *et al.* ultimately concluded that, at present, no optimal harvest site can be established (44). Instead, selection should be guided by accessibility, safety and patient preference. Nevertheless, it is imperative for surgeons to consider confounding variables such as prior local radiation therapy, surgical interventions, or liposuction, as these may compromise tissue quality (23,44).

4.2.1.2 Use of Tumescent Anesthesia

In addition to the aforementioned standard technique developed by Coleman (16), “wet” and “super wet” extraction methods were later introduced with the aim of minimizing the substantial damage to adipocytes and SVF cells that can occur during the extraction process (49). While the “wet” technique requires a comparatively smaller volume of infiltration fluid, Fodor *et al.* described the “super wet” liposuction technique, characterized by a 1:1 infiltration-to-aspiration ratio (50). It is considered to be a safe approach for larger fat removal procedures due to the reduced amount of blood loss and may also benefit AFG by enhancing graft retention through the reduction of mechanical stress during harvesting (49). Furthermore, comparative *in-vitro* studies demonstrated that tumescent anesthesia preserves a significantly greater number of viable ADSCs than local anesthesia, with higher counts of adherent ASCs after 24 hours of culture (51). However, the effect of local anesthetics, with or without vasoconstrictors, on adipocyte and SVF cell viability remains controversial, with other studies reporting both detrimental (52) and beneficial (53) outcomes. Contrary to the conclusions drawn by Moor *et al.* in previous studies (54), Weichman *et al.* discovered that local anesthetics do not have a significant impact on the long-term survival of fat grafts (55). Despite the existence of *in-vitro* evidence of its dose-dependent toxicity on fibroblasts and ADSCs (56–58), Lidocaine remains the most prevalent anesthetic agent employed in AFG (56).

4.2.1.3 Harvesting Cannula

The diameter and number of perforations in the harvesting cannula are pivotal in determining the success of fat grafting procedures (42). As demonstrated by Campbell *et al*., an inverse correlation was identified between the degree of cellular damage and the diameter of the instrument utilized for the extraction of fat (59). Numerous studies have shown that multi-perforated cannulas have the capacity to reduce local pressure at each port, thus minimizing tissue damage during fat harvesting (60). The utilization of smaller-diameter cannulas has been hypothesized to facilitate access to more superficial and vascularized adipose layers, with the potential to enhance patient comfort and the yield of ADSCs. However, findings on optimal cannula diameter remain inconsistent, with reports suggesting advantages for 2 mm, 3 mm, 4 mm and even 6 mm instruments in terms of adipocyte viability, density and graft survival (61–64). Despite the absence of a consensus on the optimal cannula diameter, it is widely accepted that the instrument should be sufficiently large to minimize shear stress and preserve adipocytes as well as SVF cells (65–67).

4.2.1.4 Power- and/or Ultrasound-assisted Liposuction

The final stage involves selecting the harvesting technique, with current approaches including direct excision, manual syringe aspiration and suction-assisted liposuction, the latter employing various pressure levels and mechanisms for their generation (68–70). In addition, liposuction may be facilitated by other methods, including liquid-jet, ultrasound pulses or laser energy (71–73). It is postulated that the level of negative pressure applied during fat harvesting may theoretically influence the viability of adipocytes and SVF cells through shear stress (23). Furthermore, several studies evaluating manual syringe aspiration, power-assisted liposuction and ultrasound-assisted liposuction have indicated that these techniques exert differential effects on cell viability and adipocyte functionality (12). It is frequently presumed that hand-held liposuction outperforms power-assisted methods in preserving cell quantity and viability. However, findings by Keck *et al*. demonstrated no statistically significant differences between these techniques (70).

Power-assisted liposuction remains a prevalent technique in AFG, yet recent evidence suggests that ultrasound-assisted liposuction is a viable alternative. Comparative *in-vitro* investigations utilizing human SAT obtained via both modalities, demonstrated that ultrasound-assisted harvesting can yield cellular preparations that are comparable to those produced by power-assisted procedures. Key outcome parameters, including adipocyte viability, total cell yield, expression of characteristic surface markers and maintenance of phenotypic stability, demonstrated no significant differences between the techniques. In addition, ADSCs isolated from ultrasound-assisted lipoaspirates exhibited proliferation rates and differentiation capabilities that were equivalent to those from power-assisted samples (74). Further independent analyses, with a particular focus on adipocyte viability within ultrasound-derived graft material, provide additional support for the potential of this method for clinical use in AFG. Collectively, these findings suggest that ultrasound-assisted liposuction not only matches the cellular quality obtained with power-assisted approaches, but may also expand the range of harvesting options without compromising regenerative potential (75). Nevertheless, a consensus on a gold standard for harvesting as a part of AFG remains to be found, with the final result being significantly influenced by further processing of the fat graft.

4.2.2 Processing

The predominant techniques for fat graft preparation include sedimentation, centrifugation, washing and filtration. The processing of the harvested lipoaspirate is of paramount importance, as it contains not only adipocytes but also collagen fibers, residual blood and cellular debris. These non-adipose components have the capacity to trigger inflammatory responses at the recipient site, with the potential to compromise graft survival. Residual blood should be removed because it accelerates the degradation of the transplanted adipose tissue. Conversely, the presence of debris can create a misleading perception of augmentation volume, as it is rapidly resorbed within hours after transplantation (24,43). In a survey conducted among members of the American Society of Plastic Surgeons in 2013, a notable variation in fat processing techniques for AFG in breast surgery was

observed. The most frequently reported method was gravity separation, applied by 45% of respondents. This was followed by centrifugation combined with filtration, applied in 34% of cases. The utilization of gauze rolling was reported by 11% of surgeons, while 7% reported employing alternative, unspecified approaches. It was observed that a minority of only 3% of all practitioners proceeded without any processing of the harvested lipoaspirate (76). A special processing approach has been developed that allows higher concentrations of SVF cells and ADSCs to be achieved in the fat graft, with the potential to improve tissue uptake and reduce resorption rates (77). This methodology is the primary focus of this work and will be discussed in greater detail in the following chapters. Firstly, an examination will be conducted into the manner, in which the respective processing techniques are executed.

4.2.2.1 Gravity Separation, Decantation and Sedimentation

The most common techniques employed in the processing of lipoaspirates are gravity separation, decantation and sedimentation (76). It has been demonstrated that, over time, this method enables spontaneous stratification of the harvested material into three distinct layers: an upper lipid-rich fraction, a middle oily phase and a lower aqueous layer. After the separation process, the oil and aqueous layers are disposed of and solely the purified fatty fraction is prepared for reinjection at the recipient site. The development of modern sterile, closed-system devices has enabled the streamlining of this process, facilitating the removal of the aqueous phase through a bottom outlet while maintaining the fat and oil fractions above. The purpose of these single-use systems is twofold: firstly, to facilitate ease of handling and secondly to contribute to reduced procedural times in AFG (24,78). Notwithstanding the aforementioned advantages, concerns have been raised regarding the potential for inaccurately high injection volumes if incomplete separation results in dilution of the graft with oil, fluid or debris (78). An *in-vitro* study by Fang *et al.* of various fat processing methods has indicated that gravity-based approaches yield the lowest proportion of pure adipose tissue, although they produce the highest total aspirate volumes. This discrepancy is likely attributable to

the retention of non-adipose components, such as residual oil, liquid and particulate matter, within the final graft material (79).

4.2.2.2 Centrifugation

First introduced by Coleman *et al.* in 1987 (16), centrifugation has since become a standard feature in the field (76). In its original form, the method involved the processing of lipoaspirate at a high speed of 3.000 revolutions per minute (rpm) for a period of 3 minutes, with the objective of separating its components. The lower layer, containing the tumescent solution and blood, is then removed. Any remaining surface oil is poured off and absorbed using a cotton pad over the course of another 3 minutes (16,78). While the core steps of the Coleman technique remain widely regarded as the gold standard for fat processing via centrifugation, more recent studies have proposed modifications to the exact centrifugation parameters (43). Nevertheless, despite the exploration of numerous centrifuge configurations, no optimal methodology has been identified to date (78).

Centrifugation has been demonstrated to enhance the concentration of progenitor cells within the processed fat (80). It is hypothesized that such concentrated lipoaspirates enhance graft survival and reduce resorption rates, likely through a vasculogenic mechanism mediated by the increased presence of those cells and associated vasculogenic factors (81,82). Conversely, while centrifugation effectively enriches cell concentrations, concerns have been raised that the forces involved may potentially damage adipocytes and ADSCs, thereby decreasing the viable count of each cell type and, in theory, reducing the success rate of fat grafting (15,83). However, an *in-vitro* experiment yielded evidence that cell viability was comparable at both 500 rpm and 1.300 rpm, despite the higher speed resulting in greater peripheral cell damage. Notwithstanding this observation, the authors recommended 1.300 rpm for *in-vivo* use, as no graft resorption was detected one year after transplantation (84). This finding is consistent with the results of several other trials that reported no significant influence of centrifugation settings on the quality or viability of lipoaspirates (46,85). However, it is challenging to draw definitive conclusions from the available data, due to the significant variation in study

designs and the absence of standardized reporting for centrifugal speed, force, duration and equipment settings (78).

4.2.2.3 Washing and Filtration

Lipoaspirate can be processed by washing, filtration, or a combination of both, typically within a closed system (78). According to the findings of a survey conducted by the American Society of Plastic Surgeons, the utilization frequency of these methods is comparable to that of centrifugation (76).

The process of washing is typically repeated multiple times using normal saline or lactated Ringer's solution, while filtration employs membranes of varying pore sizes, depending on the device. The objective of both procedures, as with all processing methods, is to eliminate oil, debris and nonviable tissue while maximizing viable ADSCs and adipocytes (78). It is noteworthy that filtration is regarded by some as being less traumatic than centrifugation and more effective at eliminating free lipids and unwanted cellular components (5). Condé-Green *et al.* demonstrated that the washing of preserved cells resulted in a higher recovery of adherent stromal cells when compared with the decanting and centrifugation methods (86). This approach may hold particular utility in large-volume grafting procedures, where centrifugation is less feasible (5).

Metal sieve filtration involves leaving fat on a sterile sieve with gentle shaking (87,88). This method produces greater inflammation and leaves more residual oil than the subsequently discussed cotton gauze rolling, potentially compromising graft viability (88). A study by Asilian *et al*., by contrast, found comparable outcomes between facial fat grafts processed by centrifugation and those prepared with metal sieve filtration combined with saline washing, as judged by both patients and surgeons (87).

4.2.2.4 Gauze Rolling

Cotton gauze rolling is a widely utilized method for preparing harvested fat grafts, most often performed with Telfa (Medtronic) gauze, though surgical towels or 4x4 gauze pads can be used as alternatives. In this technique, the fat is rolled over the gauze with the back of an instrument, allowing the material to absorb excess oil, tumescent fluid and aqueous components, leaving behind the viable fat cells. As the process of purification progresses, the graft assumes a golden appearance (78). The process generally takes 2 – 4 minutes, which is comparable to the efficiency of centrifugation and important to limit ischemia time (80). However, it should be noted that the process may become less efficient with larger fat volumes. The advantages of this method include low cost and minimal trauma to fat particles, especially when an assistant can prepare the graft during harvesting to maximize procedural efficiency (78). Preclinical *in-vivo* studies indicate that the application of cotton gauze rolling results in the highest SVF cell counts and graft retention rates in comparison to centrifugation or filtration methods. Conversely, the underlying mechanism that enables this potential advantage, in addition to its definitive superiority over alternative processing methods, remains to be elucidated (77).

4.2.3 Recipient Site Preparation

While many authors regard recipient site tissue integrity and environment as key factors in fat graft survival, the evidence remains inconclusive (12,33,38). In a recent review, Oranges *et al.* identified five major approaches for the preparation of the recipient site (89). The overarching objective of these approaches is to enhance oxygenation and nutrient supply, thereby optimizing conditions for autologous tissue grafting (8). The ensuing paragraphs therefore are to be devoted to the following subjects: external volume expansion, administration of proliferative agents such as vascular endothelial growth factor (VEGF), SVF or interleukin-8 (IL-8), induction of iatrogenic ischemia, microneedling and implantation of alloplastic materials (89).

4.2.3.1 External Volume Expansion

External volume expansion (EVE), achieved through negative pressure, has been shown to stimulate tissue changes that are favorable for fat grafting (90–92). In clinical settings, EVE is commonly performed with devices such as the BRAVA system (Brava LLC, Miami, USA). This bra-like device is utilized to apply a low vacuum pressure of up to -80 mmHg and is typically worn for 10 – 24 hours per day for 2 – 4 weeks prior to surgery (92). In preclinical *in-vivo* studies, pressures ranging from -25 mmHg to -125 mmHg have been observed to induce an increase in cell proliferation, vascularization and skin/subcutaneous thickness, with these effects being more pronounced over extended periods of application. The most commonly observed changes included enhanced adipocyte density, higher expression of proliferation markers (e.g. Ki-67, PPAR-γ), increased blood vessel density and improved skin perfusion. It has been reported in the literature that greater fat graft survival and adipocyte viability have been observed following EVE, particularly at a pressure of -125 mmHg. Although continuous pressure was employed in all animal experiments, cyclical pressure has been hypothesized to elicit more pronounced biological responses (89). In summary, applying EVE before grafting seems to create a pro-adipogenic and pro-angiogenic environment, yielding promising results with AFG take rates reported from 53% to 82% (89,92). However, further research is required to define the optimal pressure, timing and duration parameters (89).

4.2.3.2 Administration of Cell-proliferating Factors

A number of preclinical studies in mice and rats have explored the administration of cell-proliferating factors such as VEGF, SVF and IL-8 to the recipient site before or during AFG (89). VEGF, either as a standalone treatment or in combination with SVF, has been observed to induce a consistent enhancement in vascularity, adipocyte content and, in certain instances, graft weight. The most pronounced outcomes were observed in cases where both factors were co-administered (93–95). IL-8 did not significantly improve graft weight, volume or adipogenesis but was associated with reduced cyst formation (89,96). Notwithstanding the encouraging laboratory findings, there is a lack of validation

from clinical trials to date and the extent of improvements in graft weight or viability remains unconfirmed in a clinical setting (8).

4.2.3.3 Ischemia

Preliminary research conducted in a preclinical setting has indicated that the implementation of iatrogenic remote ischemic preconditioning of the recipient site may potentially enhance the survival of fat grafts by reducing the incidence of ischemia-reperfusion injury (89,97,98). In a rat model, Gassman *et al.* applied 3 5-minute cycles of temporary hindlimb ischemia before grafting, resulting in markedly higher graft viability, preserved tissue architecture and reduced fibrosis and liponecrosis compared with controls. These effects were linked to improved oxygenation and perfusion in distant capillary beds (98). Whilst this approach shows great promise, it has yet to be subjected to evaluation in clinical trials (24).

4.2.3.4 Microneedling

Preclinical studies utilizing microneedling to prepare the recipient site prior to AFG involved the ablation of subcutaneous tissue in a crisscross pattern, thereby increasing surface area (8,99,100). In rat models, both microneedle roller and 18-gauge needle abrasion increased vascularity and in some cases reduced inflammation, cyst formation and fibrosis (99,100). A study conducted by Sezgin *et al.* revealed no significant enhancement in adipocyte integrity, cell proliferation or graft viability (100). Conversely, another study by Samdal *et al.* documented modestly elevated fat survival and tissue perfusion (99). Microneedling has been shown to induce favorable vascular and inflammatory changes (100). However, the effect of microneedling on long-term graft survival remains uncertain.

4.2.3.5 Implantation of Alloplastic Material

In a rabbit model, the implantation of silicone sheets to induce capsule formation prior to autologous fat grafting resulted in increased vascularization of the recipient

site and improved graft retention. Baran *et al.* reported a 30% fat recovery after one year when grafting preserved-structure fat into preconditioned sites, compared with no residual fat in controls. However, it should be noted that this method necessitates an additional surgical session and there is a lack of human studies that have thus far evaluated its efficacy (101).

4.2.4 Grafting

The reinjection of processed lipoaspirate is a delicate step that exerts a significant influence on both aesthetic and functional outcomes (12).

4.2.4.1 Cannulas and Injection Pressure

The procedure is generally performed through a small skin incision (8). The cannulas utilized for the injection are typically smaller than those employed for harvesting and often have a single distal port, in contrast to the 2 ports characteristic of Coleman cannulas (64,65,67,102). For facial grafting, very fine cannulas (~1 mm) with varying tip shapes, diameters and lengths are used (8). Closed-system aspiration and injection devices delivering constant pressure at 11 mmHg have been shown to provide laminar fat flow, reduce the risk of local barotrauma and theoretically lower the risk of fat embolism (8).

4.2.4.2 Injection Volume

To maximize graft survival, it is recommended to inject the fat in small aliquots (1 – 2 mm) during cannula withdrawal, arranged in a fanning, crosshatch pattern at varying depths (8,16,19). This technique increases surface contact with the recipient bed, avoids localized overpressure and helps prevent central necrosis, since deposits larger than 2 – 3 mm risk developing a necrotic core once plasmatic imbibition is no longer sufficient (12,103–105). Overfilling by approximately 20% is advised to compensate for the resorption of tumescent solution in the first few postoperative days (8).

4.2.4.3 Injection Speed

The injection speed is also of critical importance. Slower rates of 0.5 – 1.0 ml/s result in smaller deposits, thereby improving oxygenation and perfusion and reducing shear-force-related cellular damage in comparison with faster rates of 3.0 – 5.0 ml/s (8,106). The selection of cannula diameter remains a subject of debate. Smaller gauges may reduce the risk of trauma and hematoma, thereby preserving oxygen delivery at the recipient site (8). Conversely, larger gauges may be more effective in preserving the integrity of adipocyte and SVF cells (65).

4.2.4.4 Injection Layers

The grafting process can be performed in a subcutaneous, intramuscular or multiple plane configuration, with the objective of expanding distribution and avoiding excessive interstitial pressure (8). Preliminary findings indicated that the intramuscular placement of the implant resulted in enhanced vascularization and improved outcomes (107). However, in specific anatomical locations, fat was observed to be more prone to mobilization in comparison to subcutaneous tissue (108). It is essential that the injection volume matches the physiological capacity of the recipient site. This principle is based on the requirement for a balanced 1:1 ratio between the transplanted fat and the recipient bed, which facilitates adequate nutrient supply and vascular ingrowth. Consequently, a breast weighing 250 g should not receive more than 250 g of grafted fat (8,109). Excessive volumes in confined areas have been demonstrated to impair perfusion, cause fat droplet coalescence into poorly integrated “lakes”, and reduce graft take (109).

Given these technical considerations, understanding the advantages and limitations of each AFG approach is essential to optimize outcomes and minimize complications.

4.2.5 Advantages and Limitations

AFG has become an increasingly popular method for soft tissue augmentation given its host compatibility, ease of harvesting, repeatability and the absence of complications such as allergic or foreign body reactions (17,91,110–112). Notwithstanding these advantages, the main challenge remains the inconsistency of long-term outcomes (113,114). The literature on this subject remains inconclusive, with reported graft survival rates showing considerable variability, ranging from below 20% to over 90% (109,115–121). Despite extensive research and growing standardization in harvesting, processing and injection techniques, these advancements have not led to a substantial reduction in clinical outcome variability (12). This suggests that patient-specific factors, particularly the number, viability and functionality of ASCs, may play a more decisive role in graft success than technique alone (122). In light of this theory, novel strategies have been devised to enhance outcomes, with Cell-assisted Lipotransfer (CAL) being a leading contender. This subject will be addressed in the subsequent section.

4.3 Cell-assisted Lipotransfer – Concept and Mechanism

Following the seminal work of Zuk *et al*. in 2002, which marked the first successful isolation of ADSCs from adipose tissue, Matsumoto *et al*. advanced the concept of CAL in 2006 (123,124). This pioneering study introduced the innovative approach of co-transplanting adipose tissue with the SVF, which contains ASCs (124). This method aimed to improve graft survival and reduce postoperative complications by leveraging the regenerative and angiogenic properties of these cells (124).

The technique was rapidly transferred from the laboratory to the clinical setting. In 2008, Yoshimura *et al.* reported the first human application of CAL, investigating its use in breast augmentation and correction of facial lipoatrophy (125). This trial demonstrated feasibility and safety, consequently initiating a series of preclinical (121,124,126–131) and clinical (115,116,132–134) studies to evaluate the efficacy of CAL in terms of volume retention, long-term survival and complication rates. Despite growing interest, study outcomes have been heterogeneous, with

some reporting clear benefits and others showing little to no difference compared to conventional AFG.

4.3.1 Mechanisms of Cell-assisted Lipotransfer

CAL is based on the principle of enriching conventional fat grafts with SVF or isolated ASCs to counteract postoperative resorption and atrophy (124). As previously described in Chapter 4.1.3, the SVF is a heterogeneous cell population obtained after enzymatic digestion of lipoaspirate and contains, besides ASCs, endothelial cells, pericytes, fibroblasts, macrophages, monocytes, lymphocytes and smooth muscle cells. These diverse cell types create a synergistic environment in which both structural and paracrine interactions can support graft survival. Within this mixture, ASCs are considered the key mediators of the regenerative effect (25–29). The yield of ASCs from adipose tissue is approximately 500-fold higher than that obtained from bone marrow, making them a particularly abundant and practical resource that is more convenient to use for AFG than the latter (135,136).

The mechanisms by which ASCs in CAL improve graft survival can be categorized into three main pathways: first, the differentiation into functional cell lineages, second, the persistence of undifferentiated stromal cells and third, paracrine signaling and secretory functions.

4.3.1.1 Differentiation into Functional Cell Lineages

ASCs are multipotent and have the capacity to differentiate into a variety of cell types, including adipocytes, endothelial cells, osteoblasts, chondrocytes, myocytes, cardiomyocytes and neurogenic cells (123,137–140). In the context of CAL, adipocyte differentiation has been shown to replenish lost fat cells within the graft, thereby directly counteracting postoperative atrophy (123,124,137,138). Furthermore, the process of differentiation into endothelial progenitors and endothelial cells promotes neovascularization, which is a critical process for maintaining graft viability by ensuring adequate blood supply and

oxygenation (141,142). This dual capacity enables ASCs to not only substitute for lost adipocytes but also to restore the vascular architecture that is essential for long-term graft integration.

4.3.1.2 Persistence of Undifferentiated Stromal Cells

A subset of transplanted ASCs remains undifferentiated within the graft. These cells provide trophic and structural support in the microenvironment (124). It is hypothesized that these cells integrate into the stromal matrix, where they maintain viability, produce extracellular matrix components and interact with other cell types. By remaining in their progenitor state, ASCs function as a reservoir of regenerative cells that are able to respond dynamically to microenvironmental cues such as hypoxia, tissue damage, or inflammatory signals. This persistence is believed to stabilize the graft during the critical early postoperative period when ischemia and necrosis are most likely to occur (36,143–145).

4.3.1.3 Paracrine Signaling and Secretory Functions

The most significant contribution of ASCs in CAL is their paracrine activity, as they have been shown to secrete a wide range of cytokines, chemokines and growth factors that enhance graft survival and integration. These include Hepatocyte Growth Factor (HGF) and Stromal Cell-Derived Factor 1 (SDF-1), which are of particular relevance due to their release in response to injury, thereby strongly promoting angiogenesis and tissue remodeling (146–149). Furthermore, ASCs have been observed to secrete Vascular Endothelial Growth Factor (VEGF), Basic Fibroblast Growth Factor (bFGF), Transforming Growth Factor-Beta (TGF- β) and Platelet-Derived Growth Factor (PDGF), all of which stimulate endothelial cell proliferation and vascular stabilization (147,150,151). Through these secretions, ASCs protect grafted fat from hypoxia-induced apoptosis and promote rapid neovascularization (123,137,138).

Moreover, the paracrine effects are not limited to angiogenesis. ADSCs also secrete immunomodulatory factors that reduce local inflammation, mitigate fibrosis and

improve integration of the transplanted tissue (152,153). By modulating immune responses, ASCs may prevent excessive scarring, pseudocyst formation, or calcification, complications that sometimes occur in conventional fat grafting (36,152,154).

4.3.1.4 Interactions of Mechanisms

It is imperative to emphasize that these mechanisms do not function in isolation. The process of differentiation into adipocytes and endothelial cells is crucial for structural regeneration. Persistent ASCs are essential for maintaining cellular reservoirs within the graft and paracrine signaling is vital for ensuring a supportive microenvironment through angiogenesis and immunomodulation (124,141,142,147). Collectively, these synergistic effects provide the scientific justification for the enhanced survival rates observed in cases of CAL, as opposed to fat grafting methodologies devoid of cellular enrichment (124).

4.3.2 Benefits and Preclinical Evidence

The efficacy of CAL in comparison with conventional AFG has been the subject of extensive investigation in preclinical studies utilizing rodent, rabbit and large animal models. Collectively, these studies provide robust experimental evidence that enrichment with SVF or ASCs enhances graft survival, vascularization and structural integrity (121,124,126–131).

The earliest preclinical work by Matsumoto *et al*. from 2006 demonstrated in severe combined immunodeficiency (SCID) mice that human CAL grafts displayed significantly larger areas of viable adipose tissue and more extensive neovascularization than conventional fat grafts, thereby introducing the concept of CAL *in-vivo* (124). This finding was subsequently corroborated through additional rodent studies. The dose-dependent effect of SVF supplementation was highlighted in 2015 when Paik *et al.* transplanted fat enriched with different cell concentrations

in nude mice. The researchers observed that graft retention exhibited a proportional enhancement with the administration of SVF cell doses up to an optimal threshold, after which further benefits were not achieved (121). In a similar vein, the optimal proportion of SVF was investigated in an athymic rat model, which confirmed that enrichment improved volumetric persistence and identified an optimal fat-to-SVF ratio of 1:1 as superior for cellular supplementation (126). Rabbit models provided further elaboration of these findings by demonstrating that grafts enriched with ASCs exhibited a significant decrease in resorption rates and increase in vascular density and histological structure in comparison to the control group (127,128). Analogous findings were also obtained in larger animal models, where the supplementation of fat grafts with ASCs or SVF in pigs resulted in improved graft volume retention. This finding serves to further enhance the translational relevance of the preclinical observations to human applications (129). As demonstrated in two preclinical studies from 2018, there is furthermore considerable potential for CAL to reduce cyst formation and fibrosis (130), as well as to enhance anti-inflammatory effects by promoting M2 macrophage polarization (131), which, in turn, is associated with increased graft retention and vascularization.

Collectively, these findings suggest that CAL consistently exhibits superior performance in preclinical models when compared with conventional fat grafting, as evidenced by enhanced graft survival, augmented angiogenesis and diminished structural degeneration. Despite the existence of disparities in methodology, animal species and enrichment strategies, the cumulative body of evidence provides substantial support for the rationale that CAL is a superior technique for soft tissue augmentation in comparison to traditional AFG. However, it is crucial to emphasize that the findings of studies utilizing animal models should not be directly extrapolated to humans. Comprehensive evaluation through clinical studies is imperative to validate these results within the clinical setting.

4.3.3 Potential Risks and Challenges

CAL was introduced with the expectation that enrichment with SVF or ASCs could not only enhance graft survival but also reduce postoperative complications (124). However, the available clinical data does not consistently confirm this assumption.

Several comparative studies have directly addressed complication rates. Peltoniemi *et al.* reported that CAL did not provide a clear advantage in terms of graft safety and showed no significant reduction of adverse events compared with standard fat grafting (132). Similarly, prospective trials by Tissiani *et al.* and Chiu *et al.* failed to demonstrate statistically significant differences in complication rates between CAL and non-CAL groups (155,156). A meta-analysis by Zhou *et al.* further concluded that, although CAL may slightly improve fat survival, it is not superior to conventional grafting in lowering the risk of complications (157). The adverse events most frequently described in these studies include pseudocyst formation, calcification and fibrosis, which remain potential outcomes of fat grafting regardless of enrichment strategy (155–157).

Overall, while CAL has shown promise in terms of fat survival, its role in reducing complications remains uncertain. Differences in methodology, such as isolation techniques, cell preparation protocols and the choice of recipient site, may partly explain the heterogeneity of reported results (132). At present, evidence does not support CAL as being clearly safer than conventional AFG regarding postoperative complications. Nevertheless, there remains a need for a more comprehensive overview of the current state of research.

4.4 Rationale for a Systematic Review

A significant number of studies have been conducted to investigate the effectiveness of CAL in terms of fat graft retention and the occurrence of postoperative complications. However, the evidence from these studies is inconsistent. Two trials by Wang *et al.* and Peltoniemi *et al.* concluded that CAL does not enhance graft survival rates (132,133). A more recent study by Vester-

Glowinski *et al.* came to the same result (158). However, a multitude of other studies have demonstrated the converse, providing evidence that CAL can achieve an enhanced survival rate compared to conventional AFG in various anatomical regions (6,115,134,144,159–162).

As previously discussed, the success of AFG depends on a multitude of factors and the variable survival rates may also be significantly influenced by the location where the procedure is performed. To derive an objective statement applicable to the general population and to minimize center bias, it is therefore appropriate to evaluate the efficacy of CAL through a systematic review. However, existing earlier systematic reviews and meta-analyses (36,143,145,157,163) concluded that further research is required in this field, which – considering the advances made in recent years – highlights the necessity of conducting an up-to-date review on CAL.

4.5 Aim

The present study is designed to contribute to a more comprehensive understanding of the clinical potential of CAL. Through a systematic comparison of CAL with conventional AFG, this work aims to improve the predictability of treatment outcomes and to generate evidence that may support the clinical implementation of CAL approaches in Austria. At present, CAL has not yet been approved for routine clinical use in Austria. The findings of this study are intended to provide a scientific foundation that may enable its future establishment in Austrian clinical practice.

In addition to its relevance for the Austrian healthcare context, this project is expected to contribute to the international effort of standardizing CAL procedures. Establishing uniform protocols and methodological consistency is regarded as a prerequisite for reproducible outcomes and ultimately for the definition of an international gold standard in clinical practice.

The primary objective of this study is the assessment of differences in fat graft take rates between CAL and conventional AFG. The secondary objective is the

evaluation and comparison of the complication rates associated with both techniques.

4.6 Hypothesis

It is hypothesized that CAL results in a superior fat graft take rate compared to conventional AFG, while not being associated with an increased incidence of postoperative complications.

5 Methods

5.1 Search Strategy

A systematic literature search was conducted in accordance with PRISMA guidelines to identify all clinical studies comparing CAL using ASCs or SVF with conventional autologous fat grafting. The electronic databases PubMed and Web of Science were searched from the date of their inception until September 30th, 2025.

The following search terms and Boolean operators were used:

("ASC" OR "adipose stem cell" OR "adipose stromal cell" OR "adipose regenerative cell" OR "SVF" OR "stromal vascular fraction" OR "ADSC" OR "adipose-derived stem cell" OR "ADRC" OR "adipose-derived regenerative cell")
AND
("fat graft" OR "lipotransfer" OR "lipofilling" OR "fat transplantation" OR "lipograft" OR "autologous fat").

Because Web of Science does not allow direct filtering for clinical trials, an additional search string was applied to restrict results to prospective human studies:

("clinical trial" OR "klinische stud*" OR "randomized controlled trial*" OR "randomised controlled trial*" OR "RCT" OR "controlled clinical trial*" OR "randomisierte kontrollierte stud*" OR "intervention stud*" OR "interventionsstud*" OR "experimental stud*" OR "experimentelle stud*" OR "multicenter stud*" OR "multizenterstud*" OR "prospective stud*" OR "prospektive stud*" OR "double blind stud*" OR "doppelblindstud*" OR "single blind stud*" OR "einfachblindstud*" OR "crossover stud*" OR "cross-over stud*" OR "crossover-stud*" OR "placebo controlled stud*" OR "placebokontrollierte stud*" OR "phase I trial*" OR "phase II trial*" OR "phase III trial*" OR "phase IV trial*").

Manual searches of reference lists and relevant review articles were performed to identify additional eligible studies not captured by database queries.

5.2 Eligibility Criteria

Studies were included if they met all of the following criteria:

- Clinical fat transplantation using SVF or ASCs as enrichment to the grafted fat
- Prospective study design with a control group receiving conventional fat grafting
- Quantitative volumetric assessment of fat survival using magnetic resonance imaging (MRI), computed tomography (CT), ultrasonography or three-dimensional (3D) surface scanning.
- Follow-up of at least three months
- Inclusion of five or more patients
- Articles written in English or German

Exclusion criteria were:

- Non-clinical (animal or *in-vitro*) studies, reviews, letters or conference abstracts
- Retrospective case series without a control group
- Studies with insufficient quantitative data or without objective volume measurement
- Duplicate publications of the same study population

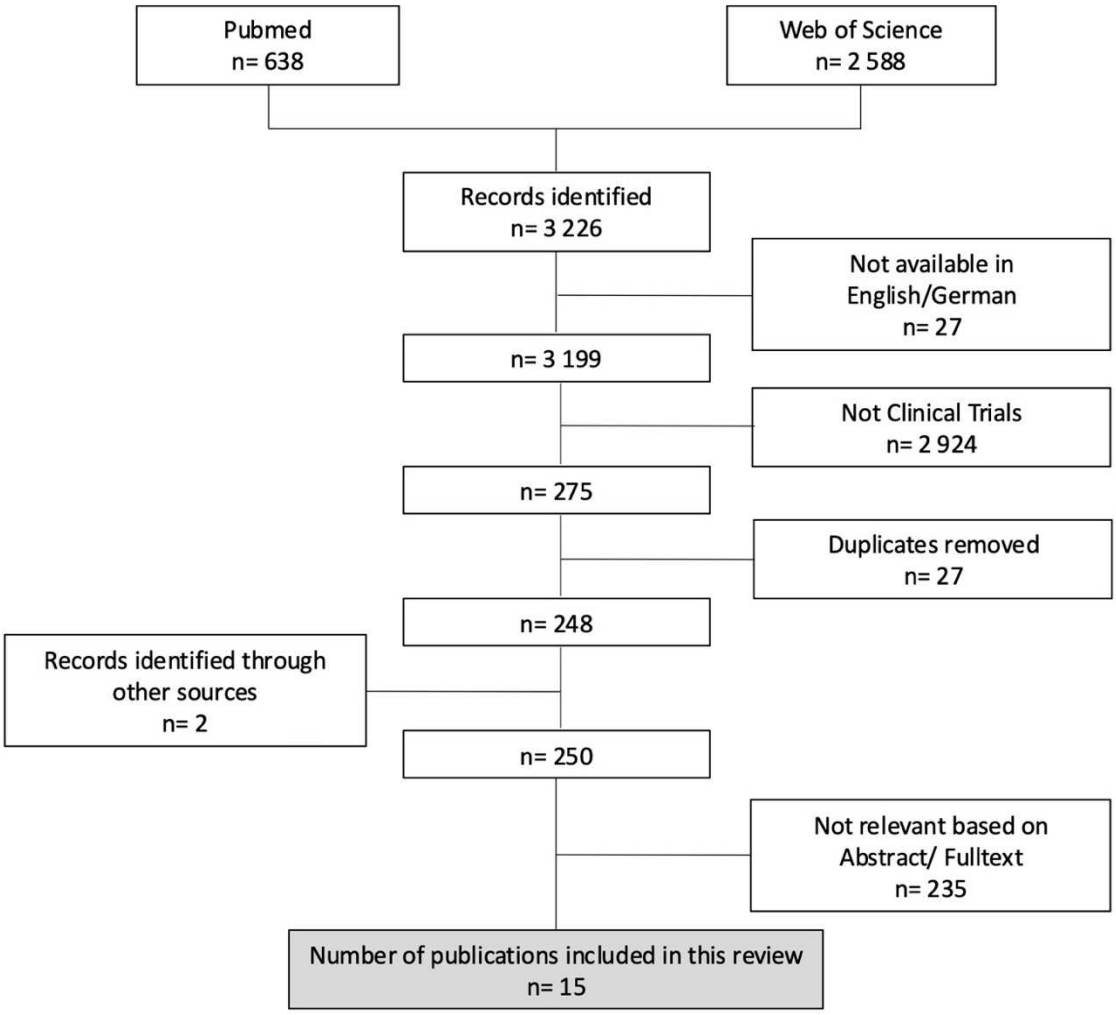
5.3 Study Selection

The selection process is illustrated in the PRISMA-style flowchart (Figure 1). The database search initially yielded 638 records from PubMed and 2559 from Web of Science, resulting in 3226 total hits. After exclusion of 27 non-English/German articles, 3199 remained. Screening for study design eliminated 2924 non-clinical trials, leaving 275 publications for further evaluation. 27 duplicates were removed, resulting in 248 unique articles, to which 2 additional studies identified by manual search were added leading to a total count of 250.

After full-text assessment, 235 articles were excluded for not meeting inclusion criteria, leaving 15 studies that were ultimately included in this systematic review.

All records were independently screened by 2 reviewers, who performed the selection and data extraction in duplicate. Any disagreements were resolved by consensus discussion.

Figure 1: Flow Diagram of the Study Selection



5.4 Data Extraction

Data were systematically extracted using a standardized template based on previous systematic reviews of CAL (36,145,157). The following parameters were recorded for each study: Author, Study Design, Level of Evidence, Intervention, Number of Patients, Age, BMI, Donor Site, Recipient Site, Number of Surgeries, Stem Cell Isolation Method, Fat Centrifugation, Mean Volume Injected, Cell Concentration, Mean Volume Gain, Volumetric Measurement Method, Fat Survival Rate and Follow-Up Months. Adverse Events, focusing exclusively on relevant complications such as oil cysts, fat necrosis, calcifications, etc. and ignoring short-term mild side effects such as swelling or redness, as well as Main Findings, including conclusions reported by the authors, were similarly recorded.

5.5 Quality Assessment

The methodological quality and level of evidence of the included studies were assessed based on the reported study design. Where explicitly stated by the authors, the level of evidence was adopted directly. In cases where no level of evidence was provided, it was assigned by the reviewers according to commonly accepted hierarchies of clinical evidence, primarily based on study design characteristics such as randomization and the presence of a control group.

Randomized controlled trials were classified as higher levels of evidence, whereas non-randomized prospective studies and controlled cohort designs were assigned accordingly lower levels. Given the scope and design of this systematic review, no formal risk-of-bias assessment tool (e.g., Cochrane Risk of Bias Tool or Newcastle-Ottawa Scale), no formal assessment of reporting bias, and no formal GRADE assessment of certainty of evidence were applied. Instead, methodological quality and the overall confidence in the evidence were evaluated narratively based on study design, sample size, consistency of findings, methodological heterogeneity, and reported outcomes.

All assessments were performed independently by 2 reviewers, with discrepancies resolved through consensus or consultation with a senior reviewer. The reliability of extracted data was ensured by cross-checking values against the original text and figures of each publication.

5.6 Data Synthesis

Given the heterogeneity in study design, stem-cell isolation methods, recipient sites and volumetric measurement techniques, no quantitative meta-analysis was performed. Instead, the data were synthesized descriptively and tabulated to highlight trends in graft survival, methodological consistency and reported complications. Volumetric outcomes were primarily compared in relative terms (percentage fat retention or mean volume gain) and subgroup comparisons (e.g. breast vs. facial fat grafting) were described qualitatively based on the direction and magnitude of effects reported across studies.

The primary outcome measure of this review was volumetric fat graft retention (take rate), as reported by the original studies, most commonly in percentage terms. Where studies reported mean volume gain instead of percentage retention, these data were extracted and interpreted descriptively as reported by the authors. The secondary outcome was the occurrence of clinically relevant complications, including oil cysts, calcifications, palpable abnormalities and fat necrosis. Mild transient postoperative findings such as edema, ecchymosis, tenderness or redness were not considered clinically relevant complications for the primary safety synthesis.

For descriptive visualization purposes, additional data preparation steps were performed in selected cases. Where outcomes were reported as ranges rather than single values, midpoint values were calculated solely to improve comparability in the graphical presentation. Where bilateral values were reported separately, mean values were calculated for descriptive representation in the figures. No such transformations were used for statistical pooling, as no meta-analysis was performed.

6 Results

6.1 Study Selection and Characteristics

15 prospective clinical studies fulfilled the prespecified eligibility criteria (prospective human trials comparing CAL (SVF or ASCs) with conventional AFG, objective volumetric outcome by MRI/CT/ultrasound/3D imaging, minimum follow-up ≥ 3 months and a concurrent fat-only control group with ≥ 5 patients) and are presented in Table 1 (115,116,132,134,144,155,158–162,164–167). Designs included randomized controlled trials and prospective controlled cohorts, predominantly Level II evidence, complemented by smaller Level III and Level IV series.

Across studies, CAL was implemented either as SVF- or ASC-enriched fat grafting, while one study directly evaluated both methods within the same trial design (159). Follow-up durations ranged from 3 to 19 months and were variably reported as single time points (115,116,132,134,144,160,161,164,166), multiple time points within the same cohort (158,159,162,165,167), or follow-up ranges (155).

Participant characteristics were reported heterogeneously across trials but consistently reflected adult cohorts undergoing autologous fat grafting for reconstructive or aesthetic indications across different recipient sites. These comprised facial applications (115,159,160,162,164,166,167), breast augmentation/reconstruction-related applications (132,134,144,155,158,161,165), and one upper-arm model (116). The most common donor sites were the abdomen (115,116,134,144,155,158–161,164–166), followed by the thighs (144,158,161,162), hips (158,161) and flanks (165,166), reflecting typical clinical lipoharvesting practice.

All primary study outcomes are reported descriptively as provided by the original studies. For graphical visualization only, midpoint values or bilateral means were calculated in selected cases, without weighting or statistical pooling. A formal meta-analysis was not performed because substantial between study heterogeneity in

recipient sites, follow-up schedules, enrichment strategies, processing and isolation workflows, and volumetric measurement methods would have limited the validity and interpretability of a pooled quantitative estimate.

Within each included study, outcomes and methodological parameters were extracted in a standardized manner and are summarized in Table 1, which forms the basis for the qualitative synthesis and the accompanying visualizations in Figures 2 and 3. Study arms shown in square brackets in Table 1 (e.g., Platelet-rich plasma (PRP) assisted fat grafting) did not meet the predefined CAL-versus-fat comparison and were therefore not included in the primary synthesis of results. Where a study reported a single mean injected volume, this value was applied to all study arms. Where multiple values were provided, they were treated as group specific as reported. Cell concentrations, if available, were inherently CAL-specific and spanned a broad range from low single-digit to higher 2- to 3-digit values ($x10^5$ cells/ml) across individual trials. Volumetric take rate assessment relied predominantly on MRI, while CT, 3D surface imaging, and ultrasound/UBM were used in selected studies, reflecting methodological heterogeneity across the clinical literature.

Table 1: Characteristics of the Included Studies

Study	Study Design	Level of Evidence	Intervention	Nr. of Patients	Age, year (M±SD/range)	BMI kg/m² (M±SD/range)	Donor Site	Recipient Site	Stem Cell Isolation Method	Fat Centrifugation	Mean Volume Injected, ml (M±SD, range)	Cell Concentration (x10⁵ Cells/ml Fat)	Mean Volume Gain, ml (M±SD, range)	Volumetric Measurement Method	Fat Survival Rate, % (M±SD, range)	FU, months (range)	Adverse Events	Main Findings
Gentile *et al.* (2012)	PCT	III	SVF + fat	10	19-60	NA	Abdomen	Breast	Automatic	3000rpm, 3min	395.4 (80-600)	0.5	NA	MRI, ultrasound	63	12	1 patient: small cystic formation, microcalcification	SVF- and PRP-enriched fat grafts show improved volume retention in the treatment of soft tissue defects. (SIGNIFICANT)
			[PRP + fat]	[13]											[69]			
			fat	10											39			
Kolle *et al.* (2013)	PRCCT	II	ASCs + fat	10	28.4 ± 8.9	24.7 ± 2.0	Abdomen	Upper arm	Manual	3000rpm, 3min	28.2 ± 3.2 (25.9-30.5)	200	23 ± 3.4 (20.6-25.4)	MRI	80.9 ± 6.0 (76.6-85.2)	4	1 patient: excluded due to incorrect graft placement	ASC-enriched fat grafts show improved volume retention, newly formed connective tissue, lower amounts of necrosis and similar amount of vessel density and oncological risk. (SIGNIFICANT)
			fat	10							28.6 ± 3.3 (26.3-30.9)		4.7 ± 2.1 (3.2-6.2)		16.3 ± 7.2 (11.1-21.4)			
Peltoniemi *et al.* (2013)	PCT	III	SVF + fat	10	48.5 ± 7.9	24.8 ± 3.9	NA	Breast	Automatic	NA	left: 289.6 ± 88.8 right: 279.3 ± 72.1	NA	left: 147.7 ± 70.7 right: 147.1 ± 57.3	MRI	left: 49.2 ± 10.0 right: 51.8 ± 9.5 mean: 50.5	6	2 patients CAL-group: small oil cysts	SVF-enriched fat grafts do not show improved volume retention in breast augmentation, are more time consuming and expensive and have a higher risk of infection. (NOT SIGNIFICANT)
			fat	8	43.4 ± 11.1	23.5 ± 2.0					left: 303.1 ± 27.6 right: 297.5 ± 27.6		left: 156.4 ± 26.9 right: 163.5 ± 24.0		left: 52.0 ± 10.3 right: 55.1 ± 7.6 mean: 53,5		1 patient control-group: small oil cysts	
Gentile *et al.* (2014)	PCT	III	SVF + fat	10	21-69	NA	Abdomen	Face	Automatic	3000rpm, 3min	NA	5.0 ± 0.07	NA	MRI, ultrasound	63	12	no major complications	SVF- and PRP-enriched fat grafts show improved volume retention in the treatment of soft tissue defects in form of burn- and post traumatic scars. (SIGNIFICANT)
			[PRP + fat]	[10]											[69]			
			fat	10											39			
Tissiani *et al.* (2016)	PCCT	II	SVF + fat	11	49.6 ± 5.7	26.3 ± 2.4	Abdomen	Breast	Manual	335g, 2min; 750g, 5min	134.3 ± 40.9	11.1 ± 18.3	NA	MRI	78.8 ± 74.9	6-19	4 patients CAL-group: fat necrosis	SVF-enriched fat grafts show improved volume retention (NOT SIGNIFICANT)
			fat	8	49.8 ± 11.5	25.9 ± 3.5				335g, 2min	111.5 ± 22.8				51.4 ± 18.4		no major complications	
Gontijo *et al.* (2017)	PRCT	II	SVF + fat	5 [15]	[42.4 ± 10.2]	[29.9 ± 2.6]	Abdomen	Face	Manual	3000rpm, 3min	[58 ± 13.6 (45-65)]	NA	NA	CT	90.4 ± 3.4 (88-92)	12	No major complications, [ecchymosis, edema]	SVF-enriched fat grafts show improved volume retention and skin quality. (SIGNIFICANT)
			fat	5 [15]	[39.7 ± 10.4]	[27.9 ± 2.1]					[53 ± 20.9 (40-70)]				76.0 ± 9.6 (70-85)			
Jeon *et al.* (2020)	PCCT	IV	SVF + fat	10	45.6 ± 8.8	24.7 ± 3.4	Abdomen/ flanks	Breast	Automatic	1000rpm, 3min 4x3000rpm, 3min	101 ± 45.8	NA	NA	3D Scan	82.2 ± 4.5/ 73.8 ± 4.7/ 65.4 ± 8.5	1/ 6/ 12	no major complications, 1 patient each group: fat necrosis	SVF-enriched fat grafts show improved volume retention for the correction of volume deficit after breast reconstruction (SIGNIFICANT)
			fat	10	47.1 ± 6.8	22.1 ± 2.1				1000rpm, 3min	97 ± 35.3				77.0 ±6.9/ 62.2 ± 9.8/ 48.4 ± 11.1			
Kolle *et al.* (2020)	PRCCT	II	ASC + fat	6	29 .0	19.2	Abdomen/ thighs/ hips	Breast	Manual	600g, 10min	222.5 (270 -182.5)	263	NA	MRI	80.2 (66.1-124.2)	4	no major complications, both groups: swelling, bruising	ASC-enriched fat grafts show improved volume retention and are safe to use even with high concentrations of ASCs supplemented. (SIGNIFICANT)
			fat	6	32.5	20.8					260 (310.6 - 240)				45.1 (36.5-50.7)			

Characteristics and outcomes of the included prospective clinical studies comparing CAL (SVF- or ASC-enriched fat grafting) with conventional autologous fat grafting (non-CAL), including study design, treatment arms, procedural parameters, volumetric outcomes, and reported adverse events. Study arms shown in square brackets (e.g. PRP-assisted fat grafting) were not included in the primary CAL versus non-CAL analysis. Abbreviations: ASC = adipose stem cells, BMI = body mass index, CT = computed tomography, FU = follow-up, M = mean, MRI = magnetic resonance imaging, NA = not available, PCCT = prospective controlled clinical trial, PCT = prospective clinical trial, PRCCT = prospective randomized controlled clinical trial, PRCT = prospective randomized clinical trial, PRP = platelet-rich plasma, rpm = revolutions per minute, SD = standard deviation, SVF = stromal vascular fraction, UBM = ultrasound biomicroscopy.

Table 1 (continued)

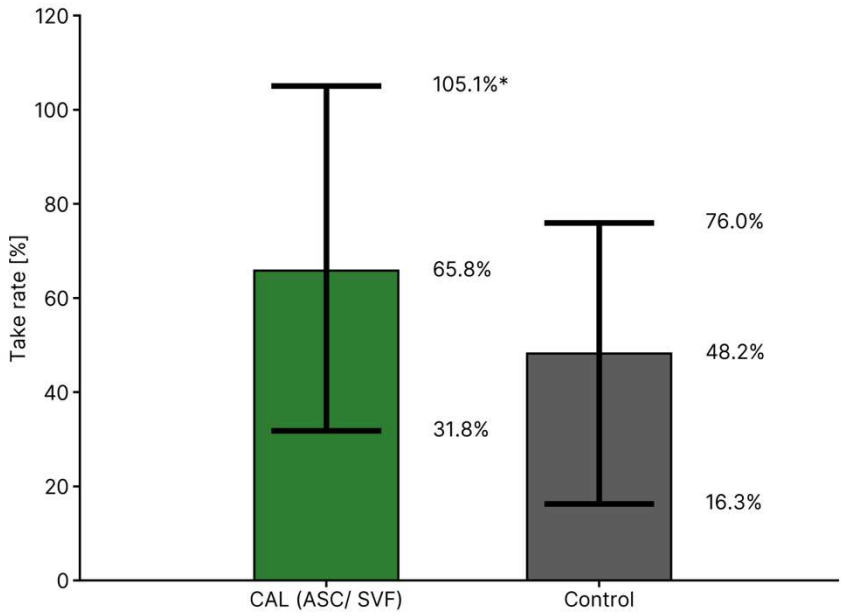
Study	Study Design	Level of Evidence	Intervention	Nr. of Patients	Age, year (M±SD/range)	BMI kg/m² (M±SD/range)	Donor Site	Recipient Site	Stem Cell Isolation Method	Fat Centrifugation	Mean Volume Injected, ml (M±SD, range)	Cell Concentration (x10⁵ Cells/ml Fat)	Mean Volume Gain, ml (M±SD, range)	Volumetric Measurement Method	Fat Survival Rate, % (M±SD, range)	FU, months (range)	Adverse Events	Main Findings
Yin *et al.* (2020)	PRCT	II	SVF + fat	25	35.5 ± 8.2	21.2 ± 1.8	Abdomen	Face	NA	NA	55.6 ± 3.8	NA	42.9 ± 5.6	3D Scan	77.6 ± 11.6	6	no major complications	SVF-enriched fat grafts show improved volume retention, wrinkles and skin texture. (SIGNIFICANT)
			fat	25	35.3 ± 8.1	21.6 ± 1.9					56.5 ± 3.8		31.7 ± 5.2		56.2 ± 9.5			
Wang *et al.* (2021)	PRCT	II	ASC + fat	6	27.4 ± 4.7	20.8 ± 1.0	Abdomen	Face	Manual	3x400g, 5min; 600g, 5min; 600g, 10 min; 600g, 5min	13 ± 2	5	NA	MRI	61.3 ± 5/ 49.8 ± 8.8	3/ 6	no major complications	ASC-enriched fat grafts show improved volume retention in the correction of facial atrophy in localized scleroderma patients. (SIGNIFICANT)
			SVF + fat	6	28.1 ± 5.2	21.9 ± 1.8				3x400g, 5min; 600g, 5min; 600g, 10 min;	14 ± 4	6			45.7 ± 10/ 31.8 ± 4.2			
			fat	6	24.4 ± 6.5	21 ± 1.5				NA	15 ± 4				41.3 ± 7.3/ 21.9 ± 4.1			
Hu *et al.* (2022)	PRCT	IV	SVF + fat	17	35.5 ± 6.9	20.3 ± 1.6	Abdomen/ thighs	Breast	Manual	2x1200rpm, 5min	Left: 272.9 ± 22.2 Right: 290.5 ± 26.2	1	NA	MRI	11 pat. >60 4 pat. >30 2 pat. <30 est. mean: 64.1	6	NA	SVF-enriched fat grafts show improved volume retention. (SIGNIFICANT)
			fat	17	34.6 ± 9.2	20.1 ± 1.9				3000rpm, 3min;	Left: 250.8 ± 21.2 Right: 268.8 ± 22.2				7 pat. >60 2 pat. >30 8 pat. <30 est. mean: 45.3			
Roshdy *et al.* (2022)	PRCT	II	ASC + fat	15 Side B	49 ± 7	≥20	Thighs	Face	Manual	NA	3.3 ± 0.2	3.6 - 6	NA	UBM	105.1 ± 71.9/ 70.9 ± 58.1	3/ 6	5 patients CAL-group: facial ecchymosis; [pain, tenderness, edema]	SVF-enriched fat grafts show improved volume retention and hypodermal thickness in temple augmentation. (SIGNIFICANT)
			fat	15 Side A											69.8 ± 60.2/ 18.9 ± 19.3		5 patients control-group: lumping at the recipient area; [pain, tenderness, edema]	
Vester-Glowinski *et al.* (2022)	PRCCT	II	ASC + fat	10 Side B	33	24.3	Abdomen/ thighs/ hips	Breast	Manual	100g, 3min	310 ± 17.6 (300-350)	100	NA	MRI	54.3/ 54 ± 32.9 (4-87)	4/ 12	both groups: increased BI-RADS score, 9 patients: oil cysts, 5 patients: not malignant palpable changes, 3 patients: fat necrosis	ASC-enriched fat grafts do not show improved volume retention in breast augmentation. (NOT SIGNIFICANT)
			fat	10 Side A											56.2/ 55.9 ± 37.8 (6-111)			
Bourne *et al.* (2023)	PCCT	II	SVF + fat	12 Side B	46.7 ± 14.1	26.9	Abdomen/ flanks	Face	Manual	1200g, 3min	37.1 ± 14.6	5.1 ± 3.4	NA	CT	50.3	9	no major complications	SVF-enriched fat grafts do not show improved volume retention. (NOT SIGNIFICANT)
			fat	12 Side A											57.3			
Wufuer *et al.* (2024)	PRCT	II	SVF + fat	11	51.64 ± 10.99	21.24 ± 2.67	NA	Face	Automatic	3000rpm, 3min	44.50 ± 4.34	15.7 ± 3.3	NA	MRI	74.5 ± 10.0/ 71.3 ±10.4	1.5/ 6	no major complications	SVF-enriched fat grafts show improved volume retention. (SIGNIFICANT)
			fat	12	49.69 ±14.20	22.84 ± 3.66									66.6 ± 13.8/ 62.0 ±13.5			

6.2 Take Rate Outcomes

Across the 15 included studies, CAL generally demonstrated higher volumetric take rate values than non-CAL at comparable follow-up intervals, although effect magnitude varied by indication and protocol. Based on the statistical interpretation reported by the original authors, 11/15 studies concluded a significantly higher take rate with CAL compared with non-CAL (115,116,134,144,159–162,164,165,167), 1 study reported a numerically higher take rate with CAL that did not reach statistical significance (155), and 3 studies reported numerically lower take rate values with CAL than with non-CAL without statistical significance (132,158,166).

These overall patterns are visually captured by the distributional summary in Figure 2 and the study- and follow-up-specific comparisons in Figure 3, which also highlight that in studies where CAL did not outperform non-CAL, the absolute differences between groups were small and the values clustered closely rather than showing a pronounced advantage for non-CAL.

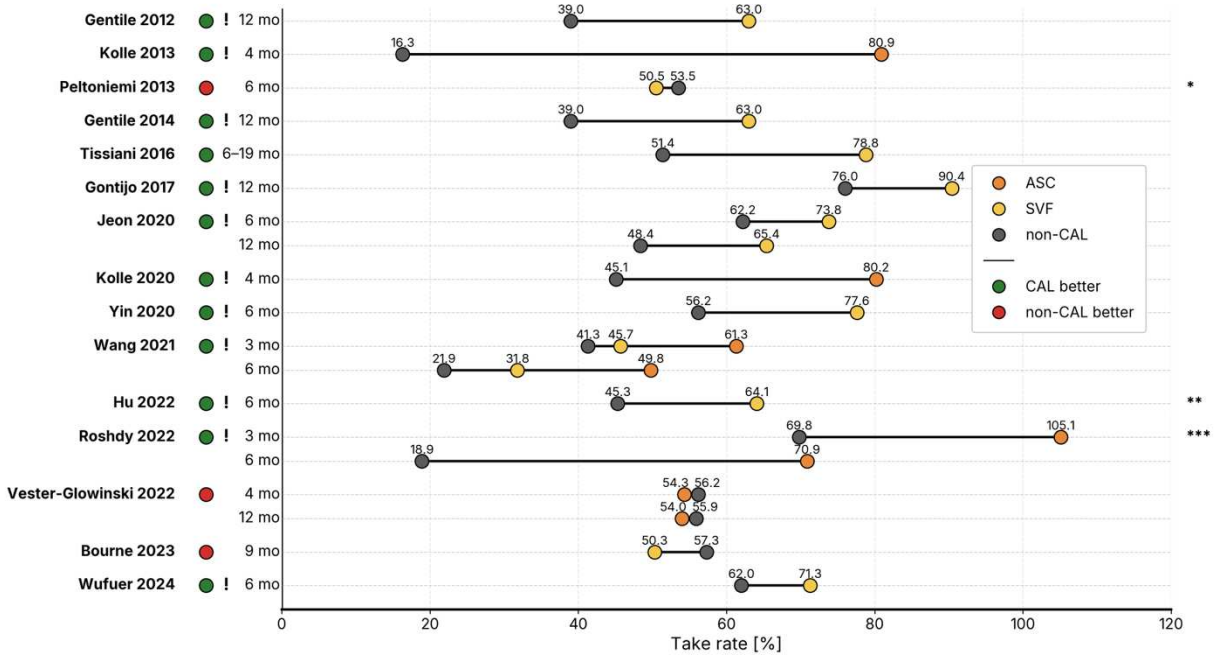
Figure 2: Mean Volumetric Outcomes (Take Rate) Across Studies



Candlestick summary of volumetric fat graft retention (take rate, %) across included studies (follow-up 3-19 months). Bars represent unweighted mean values and whiskers indicate the minimum and maximum values reported. Overall, CAL demonstrates higher mean take rates than conventional fat grafting (non-CAL), with substantial inter-study variability. Abbreviations: ASC = adipose stem cells, CAL = cell-assisted lipotransfer, SVF = stromal vascular fraction

** Values >100% due to ultrasound measurement*

Figure 3: Take Rate by Study / Follow-Up



Forest plot of study-level volumetric fat graft retention (take rate, %) comparing CAL with conventional fat grafting (non-CAL) across all included studies and follow-up intervals. CAL groups are color-coded by enrichment strategy (ASC and SVF), and horizontal lines connect treatment arms within each study. Most studies demonstrate significantly higher take rates for CAL, while differences are small and not significant in studies favoring non-CAL. Abbreviations: ASC = adipose stem cells, CAL = cell-assisted lipotransfer, SVF = stromal vascular fraction.

** Values calculated as the mean of left and right sides*
*** Values calculated as midpoints of reported ranges*
**** Values >100% due to ultrasound measurement*
! Statistically significant difference ($p < 0.05$)

6.2.1 Facial Indications

Facial studies most frequently reported higher take rate values with CAL compared to non-CAL at follow-up intervals between 3 and 12 months. Several SVF-based CAL studies demonstrated a clear numerical separation favoring CAL, such as 63.0% versus 39.0% at 12 months (164), 90.4% versus 76.0% at 12 months (115), 77.6% versus 56.2% at 6 months (160), and 71.3% versus 62.0% at 6 months (167). ASC-based CAL in temple augmentation showed marked numerical advantages at both 3 months (105.1% versus 69.8%) and 6 months (70.9% versus 18.9%), with the >100% value interpreted as modality-related overestimation in the underlying study context (ultrasound/UBM measurement) rather than true biological gain (162). A key mechanistic and comparative insight is provided by the 3-arm design in which

ASC-enriched fat, SVF-enriched fat, and non-CAL were all evaluated at the same time points. At both 3 and 6 months, take rate values followed a consistent ordering of ASC > SVF > non-CAL (61.3% > 45.7% > 41.3% at 3 months; 49.8% > 31.8% > 21.9% at 6 months) (159). Notably, where CAL did not outperform non-CAL in facial applications, differences were small and clustered closely, such as 50.5% (SVF) versus 53.5% (non-CAL) at 6 months (132), and 50.3% (SVF) versus 57.3% (non-CAL) at 9 months (166), which contrasts with the larger separations observed in multiple other facial studies.

6.2.2 Breast Indications

Breast studies showed a more heterogeneous pattern than facial studies, with both clear CAL advantages in some trials and near-overlapping outcomes in others. SVF-based CAL was numerically higher than non-CAL in several breast cohorts, including 63.0% versus 39.0% at 12 months (134), and sustained separation across multiple follow-ups in another trial (73.8% versus 62.2% at 6 months; 65.4% versus 48.4% at 12 months) (165). In a study by Hu *et al.*, take rate outcomes were reported as ranges rather than single point estimates, which is stated in Table 1. Therefore, for improved comparability and visualization in Figure 3, we calculated the midpoint of each reported range, resulting in 64.1% (SVF) versus 45.3% (non-CAL) at 6 months (144). ASC-based CAL also demonstrated notable separation in at least one breast trial, with 80.2% versus 45.1% at 4 months (161). Conversely, some breast trials reported very similar take rate values between CAL and non-CAL, such as 54.3% versus 56.2% at 4 months and 54.0% versus 55.9% at 12 months in a study conducted by Vester-Glowinski *et al.*, suggesting that any benefit may be context-dependent and potentially influenced by differences in protocols, recipient site characteristics, and measurement approaches (158). A further example of heterogeneity is seen in a trial from Peltoniemi *et al.*, where take rate values were close between groups at 6 months in a breast cohort (132). For that study, SVF take rate values were reported separately for the left and right breast (49.2% left and 51.8% right) versus non-CAL values of 52.0% left and 55.1% right. Therefore, for clearer presentation in Figure 3, the mean of both sides was calculated (SVF: 50.5% versus non-CAL: 53.5% at 6 months) (132).

6.2.3 Upper Arm Indications

A single randomized model by Kolle *et al.* evaluated CAL in an upper arm recipient site and reported higher take rate outcomes for ASC-enriched fat compared to non-CAL at 4 months (80.9% versus 16.3%) (116). Although limited to one study, this model provides a controlled within-study demonstration of a large numerical separation favoring CAL in a non-breast, non-facial recipient site (116).

6.2.4 Cross-sectional Summary at the Latest Follow-Up

Figure 2 summarizes the overall distribution of take rate outcomes across studies, showing higher mean take rate for CAL than for non-CAL when outcomes are summarized descriptively across the available follow-up windows. Figure 3 complements this by displaying the study-level values, where the majority of trials demonstrate a directional shift toward higher take rate in the CAL arm at the reported follow-up, particularly in multiple facial cohorts and several breast cohorts. Crucially, in those trials where non-CAL exceeded CAL (132,158,166), the points remain clustered in close proximity, indicating that the “non-CAL advantage” is small in magnitude compared with the larger separations seen in several CAL-favoring trials. Finally, the only direct within study comparison of ASC versus SVF supports an ordering of ASC > SVF > non-CAL at both early (3 months) and later (6 months) time points, reinforcing the interpretations that the overall CAL effect is not uniform across enrichment strategies and may depend on the specific cell product used (159).

6.3 Complication Rates

Across the included studies, clinically relevant adverse events were generally infrequent and were not uniquely associated with CAL arms. In accordance with the predefined extraction rule used in this review, mild transient postoperative symptoms, such as erythema/redness, swelling/edema, ecchymosis, tenderness, and pain, were not counted as complications even when reported by authors, as

these were short lived and not clinically meaningful adverse outcomes in the context of safety evaluation. With respect to clinically relevant complications, one study reported a small cystic formation and microcalcification in a patient (134). Oil cysts were documented in both CAL and non-CAL groups in at least one breast cohort, indicating that such events are not exclusive to CAL enrichment (132,158). Fat necrosis was reported in CAL recipients in one cohort (155) and occurred in both groups in another study (165). In a breast trial with detailed radiologic and palpable findings, increased BI-RADS scores, oil cysts, non-malignant palpable changes, and fat necrosis were reported, without the pattern being described as uniquely attributable to CAL (158). In a facial augmentation trial, ecchymosis was reported in the CAL group and lumping at the recipient area in the control group, supporting a qualitatively comparable safety profile across interventions within that study (162). Overall, most studies reported no clinically relevant complications beyond occasional low-frequency events, reflecting the generally favorable safety profile of AFG irrespective of enrichment strategy. Accordingly, CAL did not appear “more dangerous” than non-CAL within the reported follow-up intervals.

6.4 Isolation Protocols

Cell isolation workflows varied and were implemented either manually or using automated systems depending on the study. Manual processing and isolation approaches were used in multiple trials evaluating both SVF and ASC products (115,116,144,155,158,159,161,162,166). Automated systems were also used in several cohorts, illustrating that take rate improvements with CAL were not confined to a single processing paradigm (132,134,164,165,167). Reported cell concentration values, where available, spanned a wide range across studies, reflecting substantial heterogeneity in dosing strategies and reporting practices across the clinical literature. Given this heterogeneity and incomplete reporting across studies, no cross-study dose-response inference was attempted in the present synthesis.

6.5 Volumetric Measurement Methods

Volumetric measurement approaches differed across trials and included MRI, CT, 3D surface scanning, and ultrasound/UBM-based techniques. MRI was used widely across both breast and facial applications and represents the dominant modality in the included evidence (115,116,132,134,144,155,158,159,161,167). CT-based assessments were solely used in facial cohorts (115,166), whereas 3D imaging was used in both facial and breast applications as well (160,165). 1 facial study used ultrasound/UBM and reported an early take rate value exceeding 100%, which is best interpreted as a measurement artifact related to ultrasound-based quantification rather than true volume increase, particularly given the subsequent reduction at later follow-up within the same cohort (162). Across the included studies, no single volumetric measurement modality was consistently associated with superior take rate outcomes overall, suggesting that differences in reported efficacy were not driven by 1 measurement method alone.

6.6 Time-course Patterns

Across the included studies, follow-up schedules were highly heterogeneous, ranging from single assessments to repeated measurements at multiple time points between 3 and 19 months, which substantially limits direct cross-study comparability of take rate values. This is clinically relevant because volumetric retention after fat grafting is time dependent: take rate estimates are typically higher at earlier assessments and decrease with longer follow-up, such that the choice of measurement time point can meaningfully influence the apparent magnitude of treatment differences (33).

To mitigate this source of variability, this review applied a minimum follow-up threshold of ≥3 months, because early postoperative measurements may be disproportionately influenced by transient factors, whereas differences between enrichment strategies are more informative once early changes have stabilized and subsequent decline becomes apparent. Within most studies that reported repeated follow-ups, CAL remained numerically, and in many cases statistically, superior to

non-CAL across time points, although the absolute take rate values decreased over time in both arms (159,162,165). By contrast, in trials with nearly overlapping curves, similarity persisted across both early and later follow-ups (158).

Overall, the wide variation in follow-up timing represents a major driver of heterogeneity in this evidence base and further supports the descriptive synthesis approach used here. For future evidence synthesis in a meta-analytic style framework, harmonized and prespecified follow-up intervals would substantially improve between study comparability and interpretability of pooled estimates.

7 Discussion

7.1 Principal Findings

The present systematic review evaluated the clinical evidence comparing CAL with conventional AFG with respect to volumetric graft retention, complication rates, isolation protocols and volumetric measurement methods.

A central finding of this analysis is the substantial methodological heterogeneity of the available clinical evidence. In particular, direct comparison of take rate outcomes remains limited because graft retention was assessed using different volumetric techniques and reported in non-uniform ways across studies, including MRI, CT, 3D surface imaging, and ultrasound/UBM-based methods. Since these modalities differ in precision, reproducibility, and susceptibility to measurement artefacts, the reported take rate values are not fully interchangeable between studies. This heterogeneity must be considered when interpreting the overall advantage of CAL over conventional AFG.

Across the included clinical trials, the global trend indicates that CAL frequently demonstrates improved fat graft survival compared with conventional fat grafting, although the magnitude and statistical significance of this advantage vary between studies and clinical indications (115,116,134,144,159–162,164,165,167). These findings align with the general direction of previous research, where the majority of clinical investigations have reported improved long-term graft retention after CAL, while a minority of studies have found no clear benefit compared with conventional techniques (36,143,145,157,163).

CAL was originally developed to improve the survival of transplanted adipose tissue and to reduce postoperative complications associated with conventional fat grafting (124). As a supplement to standard AFG, ASCs are believed to improve graft survival through several complementary mechanisms: differentiation into adipocytes, differentiation into endothelial cells promoting angiogenesis, secretion

of growth factors supporting surrounding tissue survival, and persistence as undifferentiated stromal cells within the graft (154). Through these mechanisms, CAL has been considered a promising strategy to promote adipogenesis and vascularization within transplanted fat tissue (168).

In the majority of previously published clinical trials and experimental studies, CAL has demonstrated improved long-term fat survival compared with conventional fat grafting (115,116,134,144,159–162,164,165,167). Nevertheless, conflicting evidence exists. For example, studies from Peltoniemi *et al.*, Vester-Glowinski *et al.* and Bourne *et al.* reported no significant difference in fat survival between CAL and conventional fat grafting (132,158,166). Such discrepancies illustrate the ongoing debate regarding the clinical benefit of CAL and emphasize the importance of systematically evaluating the available evidence.

7.2 Biological Rationale of CAL

The theoretical advantage of CAL over conventional fat grafting is primarily based on the regenerative potential of ASCs and other components of the SVF. ASCs have the ability to differentiate into adipocytes and endothelial cells, thereby supporting both adipose regeneration and neovascularization. In addition, these cells release multiple cytokines and growth factors that enhance angiogenesis, tissue remodeling and resistance to ischemia in the transplanted graft (154).

Experimental studies further suggest that CAL may promote adipogenesis and angiogenesis in transplanted fat tissue, leading to improved graft survival and structural integrity. The regenerative potential of ASCs is therefore considered one of the central biological explanations for the improved volumetric outcomes reported in many CAL studies (168).

However, despite these promising biological mechanisms, clinical results remain heterogeneous. While numerous investigations have demonstrated increased fat survival after CAL, others fail to reproduce this effect. These findings may be

attributed to variations in cell isolation techniques, graft preparation methods, injected volumes and recipient site characteristics (132,133,169–171).

7.3 Influence of Recipient Site and Graft Volume

One important determinant of fat graft survival appears to be the anatomical recipient site and the associated injection volume. In general, facial fat grafting requires relatively small volumes, whereas breast augmentation or reconstruction involves substantially larger graft volumes. Large volume grafts are more susceptible to central ischemia and necrosis due to insufficient vascularization in the central region of the graft (172).

According to Yoshimura's three-zone theory, transplanted fat tissue can be divided into a survival zone, a regeneration zone and a necrotic zone. In large graft volumes, the central region is more likely to experience ischemic necrosis and subsequent resorption (172). This mechanism may partly explain why complications such as oil cyst formation, calcifications or nodules are more frequently observed in breast procedures compared with facial applications (173).

Consistent with this concept, several meta-analyses have reported that CAL improves fat retention more consistently in facial grafting than in breast augmentation procedures. Nevertheless, other studies have suggested that CAL may still provide clinical benefits in large volume grafting procedures when sufficient cell enrichment is achieved (36,157).

7.4 Role of SVF and ASC Enrichment

An important variable influencing the outcome of CAL is the concentration of stromal cells within the graft. Previous investigations have demonstrated that the dose of SVF or ASCs may directly influence fat graft survival. High dose cell enrichment has been associated with improved long-term volume retention and reduced postoperative edema. However, the optimal number of enriched cells required for

maximal graft survival remains unknown. The preparation equipment, isolation protocols and cell counting methods vary widely across studies, making direct comparison difficult. Consequently, it is currently not possible to determine the ideal cell concentration for clinical use (36,143,174).

Furthermore, the method used to isolate stromal cells may influence the clinical applicability of CAL. While enzymatic isolation techniques allow high cell yields, they require specialized laboratory facilities and may increase procedural complexity and cost. In contrast, mechanical isolation methods are faster and less expensive, but their effectiveness remains the subject of ongoing investigation (163,175).

7.5 Complications and Safety Considerations

Another crucial aspect of CAL is the safety of the technique. Most clinical studies indicate that complication rates after CAL are comparable to those observed after conventional fat grafting (156,157). The complications most frequently reported include oil cyst formation, calcifications, nodules and areas of fat necrosis (143,176,177).

Interestingly, the majority of studies did not observe a statistically significant difference in complication rates between CAL and conventional fat grafting (36,156). This finding suggests that the safety profile of AFG may depend more on procedural factors and graft volume than on the presence or absence of cellular enrichment.

A further point of discussion in the literature concerns the potential oncological safety of CAL. Experimental studies have suggested that ASCs may promote tumor growth through paracrine mechanisms (178–181). Nevertheless, clinical investigations have not demonstrated a clear increase in cancer recurrence after fat grafting procedures (157,182,183). Despite the absence of definitive evidence for increased oncological risk, caution is generally recommended when performing fat grafting procedures in patients with a history of breast cancer, particularly in the early postoperative period (184).

7.6 Limitations

This systematic review has several limitations that should be considered when interpreting the findings. No review protocol was prospectively registered, which represents a methodological limitation. In addition, no formal assessment of reporting bias was performed. Therefore, the possible influence of unpublished negative studies, selective outcome reporting, or incomplete reporting of complications cannot be excluded. Furthermore, the literature search was restricted to PubMed and Web of Science and did not include Embase or the Cochrane Library. Consequently, complete literature coverage cannot be guaranteed, and potentially relevant studies may have been missed. Beyond these review-level considerations, several study-related limitations should also be acknowledged.

First, the number of eligible studies included in the analysis was limited. Only 15 clinical studies met the predefined inclusion criteria and were therefore incorporated into the final synthesis. Although this number reflects the currently available prospective evidence directly comparing CAL with conventional AFG, the relatively small pool of studies restricts the statistical robustness and generalizability of the conclusions.

Second, the sample sizes of the individual studies were generally small. All included trials investigated relatively limited patient cohorts, with <100 patients per study. Small sample sizes inherently increase the risk of statistical variability and reduce the power to detect subtle differences between treatment approaches. Consequently, the reported outcomes may be more susceptible to random variation and may not fully reflect the true clinical effect of the intervention.

Third, considerable methodological heterogeneity was present across the included studies. Variations were observed in several procedural aspects, including fat harvesting techniques, graft processing methods, stromal vascular fraction isolation protocols, and enrichment strategies. In addition, the concentration of injected cells, preparation techniques for SVF and ASCs, and the proportion of lipoaspirate used for cell isolation differed substantially between studies. These methodological

inconsistencies complicate direct comparisons and may contribute to variability in the reported take rate outcomes.

Another important source of heterogeneity concerns the anatomical recipient sites investigated in the included trials. The studies covered a broad range of clinical indications, including facial applications, breast procedures, and upper arm augmentation. These indications differ substantially with respect to graft volume requirements, vascular environment, and tissue characteristics, all of which may influence graft survival and volumetric outcomes.

Furthermore, the follow-up intervals used to assess fat graft survival varied considerably among the included studies. As fat graft retention changes over time and tends to stabilize only after several months, differences in follow-up duration may affect the comparability of reported take rate values across studies.

Finally, the available literature does not yet provide standardized protocols for CAL procedures. Differences in cell isolation techniques, enrichment strategies, injected cell concentration, and graft preparation workflows remain common in the clinical literature. The absence of methodological standardization represents an important limitation when attempting to compare outcomes across studies and draw definitive conclusions regarding the optimal CAL protocol.

7.7 Clinical Implementation and Regulatory Considerations

Despite encouraging clinical results, the translation of CAL into routine clinical practice remains influenced not only by scientific uncertainty but also by regulatory constraints. In the European Union, cell- and tissue-based products may fall under the framework of Advanced Therapy Medicinal Products (ATMPs), which imposes specific regulatory requirements for manufacturing, quality control, traceability, and clinical use (185).

With regard to ASCs specifically, European regulatory guidance distinguishes between procedures that are considered non-substantially manipulated and those

that may qualify for stricter regulatory oversight. In particular, collagenase digestion and cell culture are regarded as substantial manipulation, whereas mechanical isolation and use in homologous subcutaneous tissue are viewed more permissively (186).

Accordingly, several ASC-based or SVF-based approaches described in the literature may be difficult to implement routinely in the EU, especially when they involve enzymatic processing, culture expansion, or non-homologous use, and some published applications have therefore been described as potentially off-label under European regulatory standards. These regulatory barriers likely contribute to the relatively slow and heterogeneous clinical development of CAL, since more complex cell-processing workflows are more difficult to standardize, validate, and integrate into routine care under current European requirements (186–188).

In this context, the continued lack of high-quality, standardized clinical evidence is not only a scientific issue but also a practical barrier to implementation, because novel stem-cell-based procedures generally require robust clinical validation before broader adoption can be justified (187).

7.8 Future Perspectives

Despite these limitations, the current body of evidence suggests that CAL remains a promising technique for improving the survival of fat grafts. Future research should focus on large prospective randomized clinical trials with standardized cell isolation protocols, clearly defined cell concentrations and objective volumetric measurement methods.

Such studies would help determine the optimal protocols for CAL and clarify whether specific enrichment strategies or recipient site characteristics influence treatment outcomes. Ultimately, improved methodological standardization will be necessary to determine the true clinical value of CAL and its role in routine clinical practice.

8 Conclusion

In summary, the available clinical evidence suggests that CAL may improve fat graft survival compared with conventional AFG. The regenerative properties of ASCs and SVF are believed to enhance angiogenesis, tissue regeneration and long-term graft retention (154).

However, the magnitude of this benefit remains inconsistent across studies. While many investigations report improved volumetric outcomes after CAL, others demonstrate no significant advantage compared with conventional techniques (132,133,157). In addition, current evidence indicates that complication rates after CAL are generally comparable to those observed after conventional fat grafting (36,156).

Overall, CAL represents a promising but not yet fully standardized approach for improving the outcomes of fat grafting procedures. Further large-scale randomized clinical trials with standardized methodologies and long-term follow-up are required to clarify the true efficacy, safety and optimal clinical indications of this technique.

9 References

The linguistic optimization of this thesis was supported by the following tools:

- ChatGPT (version GPT-5.3, OpenAI, https://chatgpt.com, 2026)
- DeepL (version 25.10.22981688, DeepL SE, https://www.deepl.com/de, 2026)

These tools were used exclusively for stylistic and grammatical improvements. All scientific content and interpretations were created and verified by the author.

1. Hoppela E, Grönroos TJ, Saarikko AM, Tervala TV, Kauhanen S, Nuutila P, et al. Fat Grafting Can Induce Browning of White Adipose Tissue. Plast Reconstr Surg - Glob Open. 2018 Jun;6(6):e1804. doi:10.1097/GOX.0000000000001804

2. Saely CH, Geiger K, Drexel H. Brown versus White Adipose Tissue: A Mini-Review. Gerontology. 2012;58(1):15–23. doi:10.1159/000321319

3. Stanford KI, Middelbeek RJW, Goodyear LJ. Exercise Effects on White Adipose Tissue: Beiging and Metabolic Adaptations. Diabetes. 2015 Jul 1;64(7):2361–8. doi:10.2337/db15-0227

4. Bucky LP, Percec I. The Science of Autologous Fat Grafting: Views on Current and Future Approaches to Neoadipogenesis.

5. Bellini E, Grieco MP, Raposio E. The science behind autologous fat grafting. Ann Med Surg. 2017 Dec;24:65–73. doi:10.1016/j.amsu.2017.11.001

6. Kølle SFT, Fischer-Nielsen A, Mathiasen AB, Elberg JJ, Oliveri RS, Glovinski PV, et al. Enrichment of autologous fat grafts with ex-vivo expanded adipose tissue-derived stem cells for graft survival: a randomised placebo-controlled trial. The Lancet. 2013 Sep;382(9898):1113–20. doi:10.1016/S0140-6736(13)61410-5

7. O'Halloran N, Courtney D, Kerin MJ, Lowery AJ. Adipose-Derived Stem Cells in Novel Approaches to Breast Reconstruction: Their Suitability for Tissue Engineering and Oncological Safety. Breast Cancer Basic Clin Res. 2017 Jan 1;11:117822341772677. doi:10.1177/1178223417726777

8. Shauly O, Gould DJ, Ghavami A. Fat Grafting: Basic Science, Techniques, and Patient Management. Plast Reconstr Surg - Glob Open. 2022 Mar 18;10(3):e3987. doi:10.1097/GOX.0000000000003987

9. E B, Jw M. Historical review and present status of free fat graft autotransplantation in plastic and reconstructive surgery. Plast Reconstr Surg. 1989 Feb;83(2). doi:10.1097/00006534-198902000-00033 PubMed PMID: 2643129.

10. NEUBER F. Fettransplantation. Chir Kongr Verhandl Dsch Gesellch Chir. 1893;22:66.

11. Czerny V. Plastischer Ersatz der Brustdrüse durch ein Lipom - New York University Health Sciences [Internet]. 1895 [cited 2025 Apr 4]. Available from: https://search.hsl.med.nyu.edu

12. Strong AL, Cederna PS, Rubin JP, Coleman SR, Levi B. The Current State of Fat Grafting: A Review of Harvesting, Processing, and Injection Techniques. Plast Reconstr Surg. 2015 Oct;136(4):897–912. doi:10.1097/PRS.0000000000001590

13. Peer LA. Loss of weight and volume in human fat grafts: With postulation of a “cell survival theory.” Plast Reconstr Surg. 1950 Mar;5(3):217.

14. Fat Grafting: Current Concept, Clinical Application, and Regenerative Potential, Part 1. Clin Plast Surg. 2015 Apr 1;42(2):ix–x. doi:10.1016/j.cps.2015.02.001

15. Zielins ER, Brett EA, Longaker MT, Wan DC. Autologous Fat Grafting: The Science Behind the Surgery. Aesthet Surg J. 2016 Apr;36(4):488–96. doi:10.1093/asj/sjw004

16. Coleman. Structural fat grafting. Aesthet Surg J. 1998 Oct;18(5). doi:10.1016/S1090-820X(98)70098-6 PubMed PMID: 19328166.

17. Coleman SR. Long-Term survival of fat transplants: Controlled demonstrations. Aesthetic Plast Surg. 1995 Sep 1;19(5):421–5. doi:10.1007/BF00453875

18. Coleman SR, Saboeiro AP. Fat Grafting to the Breast Revisited: Safety and Efficacy. Plast Reconstr Surg. 2007 Mar;119(3):775. doi:10.1097/01.prs.0000252001.59162.c9

19. Coleman SR. Facial recontouring with lipostructure. Clin Plast Surg. 1997 Apr;24(2):347–67. PubMed PMID: 9142473.

20. Coleman SR. Hand rejuvenation with structural fat grafting. Plast Reconstr Surg. 2002 Dec;110(7):1731–44; discussion 1745-1747. doi:10.1097/01.PRS.0000033936.43357.08 PubMed PMID: 12447057.

21. Doornaert M, Colle J, De Maere E, Declercq H, Blondeel P. Autologous fat grafting: Latest insights. Ann Med Surg. 2019 Jan;37:47–53. doi:10.1016/j.amsu.2018.10.016

22. Simonacci F, Bertozzi N, Grieco MP, Grignaffini E, Raposio E. Autologous fat transplantation for breast reconstruction: A literature review. Ann Med Surg. 2016 Dec;12:94–100. doi:10.1016/j.amsu.2016.11.012

23. Suszynski TM, Sieber DA, Van Beek AL, Cunningham BL. Characterization of Adipose Tissue for Autologous Fat Grafting. Aesthet Surg J. 2015 Feb 1;35(2):194–203. doi:10.1093/asj/sju059

24. Luze H. Autologous fat grafting in reconstructive breast surgery. [Graz]: Medical University of Graz; 2022.

25. Lunger A, Ismail T, Todorov A, Buergin J, Lunger F, Oberhauser I, et al. Improved Adipocyte Viability in Autologous Fat Grafting With Ascorbic Acid–Supplemented Tumescent Solution. Ann Plast Surg. 2019 Oct;83(4):464–7. doi:10.1097/SAP.0000000000001857

26. Khouri RK. Current Clinical Applications of Fat Grafting. Plast Reconstr Surg. 2017 Sep;140(3):466e–86e. doi:10.1097/PRS.0000000000003648

27. Ismail T, Bürgin J, Todorov A, Osinga R, Menzi N, Largo RD, et al. Low osmolality and shear stress during liposuction impair cell viability in autologous fat grafting. J Plast Reconstr Aesthet Surg. 2017 May;70(5):596–605. doi:10.1016/j.bjps.2017.01.023

28. Zimmerlin L, Donnenberg VS, Pfeifer ME, Meyer EM, Péault B, Rubin JP, et al. Stromal vascular progenitors in adult human adipose tissue. Cytometry A. 2010 Jan;77A(1):22–30. doi:10.1002/cyto.a.20813

29. Kanthilal M, Darling EM. Characterization of Mechanical and Regenerative Properties of Human, Adipose Stromal Cells. Cell Mol Bioeng. 2014 Dec;7(4):585–97. doi:10.1007/s12195-014-0350-y

30. Rosen ED, MacDougald OA. Adipocyte differentiation from the inside out. Nat Rev Mol Cell Biol. 2006 Dec 1;7(12):885–96. doi:10.1038/nrm2066

31. Pu LLQ. Mechanisms of Fat Graft Survival. Ann Plast Surg. 2016 Aug;77(Supplement 1):S84–6. doi:10.1097/SAP.0000000000000730

32. Peer LA. CELL SURVIVAL THEORY VERSUS REPLACEMENT THEORY. Plast Reconstr Surg. 1955 Sep;16(3):161.

33. Shih L, Davis MJ, Winocour SJ. The Science of Fat Grafting. Semin Plast Surg. 2020 Feb;34(01):005–10. doi:10.1055/s-0039-3402073

34. Suga H, Eto H, Aoi N, Kato H, Araki J, Doi K, et al. Adipose Tissue Remodeling under Ischemia: Death of Adipocytes and Activation of Stem/Progenitor Cells: Plast Reconstr Surg. 2010 Dec;126(6):1911–23. doi:10.1097/PRS.0b013e3181f4468b

35. Yoshimura K, Eto H, Kato H, Doi K, Aoi N. In vivo Manipulation of Stem Cells for Adipose Tissue Repair/Reconstruction. Regen Med. 2011 Nov;6(sup6):33–41. doi:10.2217/rme.11.62

36. Zhao J, Chen J, Xu C, Yang S, Guo S, Zhou B. The efficacy of cell-assisted versus conventional lipotransfer: A systematic review and meta-analysis. Asian J Surg. 2023 Jan;46(1):35–46. doi:10.1016/j.asjsur.2022.04.031

37. Rasmussen BS, Lykke Sørensen C, Vester-Glowinski PV, Herly M, Trojahn Kølle SF, Fischer-Nielsen A, et al. Effect, Feasibility, and Clinical Relevance of Cell Enrichment in Large Volume Fat Grafting: A Systematic Review. Aesthet Surg J. 2017 Jul 1;37(suppl_3):S46–58. doi:10.1093/asj/sjx008

38. Luze H, Schwarz A, Philipp Nischwitz S, Kolb D, Bounab K, Zrim R, et al. Autologous Fat Grafting in Reconstructive Breast Surgery: Clinically Relevant Factors Affecting the Graft Take. Aesthet Surg J. 2022 Dec 14;42(12):NP745–55. doi:10.1093/asj/sjac166

39. Egro FM, Roy E, Rubin JP, Coleman SR. Evolution of the Coleman Technique. Plast Reconstr Surg. 2022 Aug;150(2):329e–36e. doi:10.1097/PRS.0000000000009355

40. Gabriel A, Champaneria MC, Maxwell GP. Fat grafting and breast reconstruction: tips for ensuring predictability. Gland Surg. 2015;4(3).

41. O'Halloran N, Potter S, Kerin M, Lowery A. Recent Advances and Future Directions in Postmastectomy Breast Reconstruction. Clin Breast Cancer. 2018 Aug;18(4):e571–85. doi:10.1016/j.clbc.2018.02.004

42. Fontes T, Brandão I, Negrão R, Martins MJ, Monteiro R. Autologous fat grafting: Harvesting techniques. Ann Med Surg. 2018 Dec;36:212–8. doi:10.1016/j.amsu.2018.11.005

43. Simonacci F, Bertozzi N, Grieco MP, Grignaffini E, Raposio E. Procedure, applications, and outcomes of autologous fat grafting. Ann Med Surg. 2017 Aug;20:49–60. doi:10.1016/j.amsu.2017.06.059

44. Li K, Gao J, Zhang Z, Li J, Cha P, Liao Y, et al. Selection of Donor Site for Fat Grafting and Cell Isolation. Aesthetic Plast Surg. 2013 Feb;37(1):153–8. doi:10.1007/s00266-012-9991-1

45. Ullmann Y, Shoshani O, Fodor A, Ramon Y, Carmi N, Eldor L, et al. Searching for the Favorable Donor Site for Fat Injection: In Vivo Study Using the Nude Mice Model. Dermatol Surg. 2006 Mar 21;31(10):1304–7. doi:10.1111/j.1524-4725.2005.31207

46. Rohrich RJ, Sorokin ES, Brown SA. In Search of Improved Fat Transfer Viability: A Quantitative Analysis of the Role of Centrifugation and Harvest Site: Plast Reconstr Surg. 2004 Jan;113(1):391–5. doi:10.1097/01.PRS.0000097293.56504.00

47. Padoin AV, Braga-Silva J, Martins P, Rezende K, Rezende ARDR, Grechi B, et al. Sources of Processed Lipoaspirate Cells: Influence of Donor Site on Cell Concentration: Plast Reconstr Surg. 2008 Aug;122(2):614–8. doi:10.1097/PRS.0b013e31817d5476

48. Geissler PJ, Davis K, Roostaeian J, Unger J, Huang J, Rohrich RJ. Improving Fat Transfer Viability: The Role of Aging, Body Mass Index, and Harvest Site. Plast Reconstr Surg. 2014 Aug;134(2):227–32. doi:10.1097/PRS.0000000000000398

49. Klein JA. Tumescent technique for local anesthesia improves safety in large-volume liposuction. Plast Reconstr Surg. 1993 Nov;92(6):1085–98; discussion 1099-1100. PubMed PMID: 8234507.

50. Fodor PB. Editorial. Wetting solutions in aspirative lipoplasty: A plea for safety in liposuction. Aesthetic Plast Surg. 1995 Jul 1;19(4):379–80. doi:10.1007/BF00451665

51. Wang WZ, Fang XH, Williams SJ, Stephenson LL, Baynosa RC, Khiabani KT, et al. Lidocaine-Induced ASC Apoptosis (Tumescent vs. Local Anesthesia). Aesthetic Plast Surg. 2014 Oct;38(5):1017–23. doi:10.1007/s00266-014-0387-2

52. Keck M, Zeyda M, Gollinger K, Burjak S, Kamolz LP, Frey M, et al. Local Anesthetics Have a Major Impact on Viability of Preadipocytes and Their Differentiation into Adipocytes: Plast Reconstr Surg. 2010 Nov;126(5):1500–5. doi:10.1097/PRS.0b013e3181ef8beb

53. Agostini T, Lazzeri D, Pini A, Marino G, Li Quattrini A, Bani D, et al. Wet and Dry Techniques for Structural Fat Graft Harvesting: Histomorphometric and Cell Viability Assessments of Lipoaspirated Samples. Plast Reconstr Surg. 2012 Aug;130(2):331e–9e. doi:10.1097/PRS.0b013e3182589f76

54. Moore JH, Kolaczynski JW, Morales LM, Considine RV, Pietrzkowski Z, Noto PF, et al. Viability of fat obtained by syringe suction lipectomy: effects of local anesthesia with lidocaine. Aesthetic Plast Surg. 1995 Jul;19(4):335–9. doi:10.1007/BF00451659

55. Weichman KE, Warren SM. Effects of Lidocaine Plus Epinephrine and Prilocaine on Autologous Fat Graft Survival.

56. Gugerell A, Kober J, Schmid M, Nickl S, Kamolz LP, Keck M. Botulinum toxin A and lidocaine have an impact on adipose-derived stem cells, fibroblasts, and mature adipocytes in vitro. J Plast Reconstr Aesthet Surg. 2014 Sep;67(9):1276–81. doi:10.1016/j.bjps.2014.05.029

57. Grambow F, Rutkowski R, Podmelle F, Schmoeckel K, Siegerist F, Domanski G, et al. The Impact of Lidocaine on Adipose-Derived Stem Cells in Human Adipose Tissue Harvested by Liposuction and Used for Lipotransfer. Int J Mol Sci. 2020 Apr 20;21(8):2869. doi:10.3390/ijms21082869

58. Kubrova E, Su M, Galeano-Garces C, Galvan ML, Jerez S, Dietz AB, et al. Differences in Cytotoxicity of Lidocaine, Ropivacaine, and Bupivacaine on the Viability and Metabolic Activity of Human Adipose-Derived Mesenchymal Stem Cells. Am J Phys Med Rehabil. 2021 Jan;100(1):82–91. doi:10.1097/PHM.0000000000001529

59. Campbell GLeM, Laudenslager N, Newman J. The Effect of Mechanical Stress on Adipocyte Morphology and Metabolism. Am J Cosmet Surg. 1987 Jun;4(2):89–94. doi:10.1177/074880688700400202

60. Hivernaud V, Lefourn B, Guicheux J, Weiss P, Festy F, Girard AC, et al. Autologous Fat Grafting in the Breast: Critical Points and Technique Improvements. Aesthetic Plast Surg. 2015 Aug;39(4):547–61. doi:10.1007/s00266-015-0503-y

61. Trivisonno A, Di Rocco G, Cannistra C, Finocchi V, Torres Farr S, Monti M, et al. Harvest of Superficial Layers of Fat With a Microcannula and Isolation of Adipose Tissue-Derived Stromal and Vascular Cells. Aesthet Surg J. 2014 May 1;34(4):601–13. doi:10.1177/1090820X14528000

62. Alharbi Z, Opländer C, Almakadi S, Fritz A, Vogt M, Pallua N. Conventional vs. micro-fat harvesting: How fat harvesting technique affects tissue-engineering approaches using adipose tissue-derived stem/stromal cells. J Plast Reconstr Aesthet Surg. 2013 Sep;66(9):1271–8. doi:10.1016/j.bjps.2013.04.015

63. Rubino C, Mazzarello V, Faenza M, Montella A, Santanelli F, Farace F. A Scanning Electron Microscope Study and Statistical Analysis of Adipocyte Morphology in Lipofilling: Comparing the Effects of Harvesting and Purification Procedures With 2 Different Techniques. Ann Plast Surg. 2015 Jun;74(6):718–21. doi:10.1097/SAP.0b013e3182a1e5a4

64. Erdim M, Tezel E, Numanoglu A, Sav A. The effects of the size of liposuction cannula on adipocyte survival and the optimum temperature for fat graft storage: an experimental study. J Plast Reconstr Aesthet Surg. 2009 Sep;62(9):1210–4. doi:10.1016/j.bjps.2008.03.016

65. Ozsoy Z, Kul Z, Bilir A. The role of cannula diameter in improved adipocyte viability: A quantitative analysis. Aesthet Surg J. 2006 May;26(3):287–9. doi:10.1016/j.asj.2006.04.003

66. Gause TM, Kling RE, Sivak WN, Marra KG, Rubin JP, Kokai LE. Particle size in fat graft retention: A review on the impact of harvesting technique in lipofilling surgical outcomes. Adipocyte. 2014 Oct 2;3(4):273–9. doi:10.4161/21623945.2014.957987

67. Kirkham JC, Lee JH, Medina MA, McCormack MC, Randolph MA, Austen WG. The Impact of Liposuction Cannula Size on Adipocyte Viability. Ann Plast Surg. 2012 Oct;69(4):479–81. doi:10.1097/SAP.0b013e31824a459f

68. Smith P, Adams WP, Lipschitz AH, Chau B, Sorokin E, Rohrich RJ, et al. Autologous Human Fat Grafting: Effect of Harvesting and Preparation Techniques on Adipocyte Graft Survival: Plast Reconstr Surg. 2006 May;117(6):1836–44. doi:10.1097/01.prs.0000218825.77014.78

69. Barzelay A, Levy R, Kohn E, Sella M, Shani N, Meilik B, et al. Power-Assisted Liposuction Versus Tissue Resection for the Isolation of Adipose Tissue–Derived Mesenchymal Stem Cells: Phenotype, Senescence, and Multipotency at Advanced Passages. Aesthet Surg J. 2015 Sep;35(7):NP230–40. doi:10.1093/asj/sjv055

70. Keck M, Kober J, Riedl O, Kitzinger HB, Wolf S, Stulnig TM, et al. Power assisted liposuction to obtain adipose-derived stem cells: Impact on viability and differentiation to adipocytes in comparison to manual aspiration. J Plast Reconstr Aesthet Surg. 2014 Jan;67(1):e1–8. doi:10.1016/j.bjps.2013.08.019

71. Shridharani S, Broyles J, Matarasso A. Liposuction devices: technology update. Med Devices Evid Res. 2014 Jul;241. doi:10.2147/MDER.S47322

72. Chung MT, Zimmermann AS, Paik KJ, Morrison SD, Hyun JS, Lo DD, et al. Isolation of Human Adipose-Derived Stromal Cells Using Laser-Assisted Liposuction and Their Therapeutic Potential in Regenerative Medicine. Stem Cells Transl Med. 2013 Oct 1;2(10):808–17. doi:10.5966/sctm.2012-0183

73. Yin S, Luan J, Fu S, Wang Q, Zhuang Q. Does Water-Jet Force Make a Difference in Fat Grafting? In Vitro and In Vivo Evidence of Improved Lipoaspirate Viability and Fat Graft Survival: Plast Reconstr Surg. 2015 Jan;135(1):127–38. doi:10.1097/PRS.0000000000000780

74. Duscher D, Atashroo D, Maan ZN, Luan A, Brett EA, Barrera J, et al. Ultrasound-Assisted Liposuction Does Not Compromise the Regenerative Potential of Adipose-Derived Stem Cells. Stem Cells Transl Med. 2016 Feb 1;5(2):248–57. doi:10.5966/sctm.2015-0064

75. Schafer ME, Hicok KC, Mills DC, Cohen SR, Chao JJ. Acute Adipocyte Viability After Third-Generation Ultrasound-Assisted Liposuction. Aesthet Surg J. 2013 Jul 1;33(5):698–704. doi:10.1177/1090820X13485239

76. Kling RE, Mehrara BJ, Pusic AL, Young VL, Hume KM, Crotty CA, et al. Trends in Autologous Fat Grafting to the Breast: A National Survey of the American Society of Plastic Surgeons. Plast Reconstr Surg. 2013 Jul;132(1):35–46. doi:10.1097/PRS.0b013e318290fad1

77. Fisher C, Grahovac TL, Schafer ME, Shippert RD, Marra KG, Rubin JP. Comparison of Harvest and Processing Techniques for Fat Grafting and Adipose Stem Cell Isolation: Plast Reconstr Surg. 2013 Aug;132(2):351–61. doi:10.1097/PRS.0b013e3182958796

78. Xue EY, Narvaez L, Chu CK, Hanson SE. Fat Processing Techniques. Semin Plast Surg. 2020 Feb;34(01):011–6. doi:10.1055/s-0039-3402052

79. Fang C, Patel P, Li H, Huang LT, Wan H, Collins S, et al. Physical, Biochemical, and Biologic Properties of Fat Graft Processed via Different Methods. Plast Reconstr Surg - Glob Open. 2020 Aug;8(8):e3010. doi:10.1097/GOX.0000000000003010

80. Canizares O, Thomson JE, Allen RJ, Davidson EH, Tutela JP, Saadeh PB, et al. The Effect of Processing Technique on Fat Graft Survival. Plast Reconstr Surg. 2017 Nov;140(5):933–43. doi:10.1097/PRS.0000000000003812

81. Allen RJ, Canizares O, Scharf C, Nguyen PD, Thanik V, Saadeh PB, et al. Grading Lipoaspirate: Is There an Optimal Density for Fat Grafting? Plast Reconstr Surg. 2013 Jan;131(1):38–45. doi:10.1097/PRS.0b013e3182729cc6

82. Sarfati I, Van La Parra RFD, Terem-Rapoport CA, Benyahi D, Nos C, Clough KB. A prospective randomized study comparing centrifugation and sedimentation for fat grafting in breast reconstruction. J Plast Reconstr Aesthet Surg. 2017 Sep;70(9):1218–28. doi:10.1016/j.bjps.2017.06.010

83. Ross RJ, Shayan R, Mutimer KL, Ashton MW. Autologous Fat Grafting: Current State of the Art and Critical Review. Ann Plast Surg. 2014 Sep;73(3):352–7. doi:10.1097/SAP.0b013e31827aeb51

84. Ferraro GA, De Francesco F, Tirino V, Cataldo C, Rossano F, Nicoletti G, et al. Effects of a New Centrifugation Method on Adipose Cell Viability for Autologous Fat Grafting. Aesthetic Plast Surg. 2011 Jun;35(3):341–8. doi:10.1007/s00266-010-9613-8

85. Pulsfort AK, Wolter TP, Pallua N. The Effect of Centrifugal Forces on Viability of Adipocytes in Centrifuged Lipoaspirates: Ann Plast Surg. 2011 Mar;66(3):292–5. doi:10.1097/SAP.0b013e3181c7140e

86. Condé-Green A, Gontijo De Amorim NF, Pitanguy I. Influence of decantation, washing and centrifugation on adipocyte and mesenchymal stem cell content of aspirated adipose tissue: A comparative study. J Plast Reconstr Aesthet Surg. 2010 Aug;63(8):1375–81. doi:10.1016/j.bjps.2009.07.018

87. Asilian A, Siadat AH, Iraji R. Comparison of fat maintenance in the face with centrifuge versus filtered and washed fat. J Res Med Sci. 2014.

88. Minn KW, Min KH, Chang H, Kim S, Heo EJ. Effects of Fat Preparation Methods on the Viabilities of Autologous Fat Grafts. Aesthetic Plast Surg. 2010 Oct;34(5):626–31. doi:10.1007/s00266-010-9525-7

89. Oranges CM, Striebel J, Tremp M, Madduri S, Kalbermatten DF, Harder Y, et al. The Preparation of the Recipient Site in Fat Grafting: A Comprehensive Review of the Preclinical Evidence. Plast Reconstr Surg. 2019 Apr;143(4):1099–107. doi:10.1097/PRS.0000000000005403

90. Khouri R, Vecchio DD. Breast Reconstruction and Augmentation Using Pre-Expansion and Autologous Fat Transplantation. Clin Plast Surg. 2009 Apr 1;36(2):269–80. doi:10.1016/j.cps.2008.11.009 PubMed PMID: 19309653.

91. Khouri RK, Eisenmann-Klein M, Cardoso E, Cooley BC, Kacher D, Gombos E, et al. Brava and Autologous Fat Transfer Is a Safe and Effective Breast Augmentation Alternative: Results of a 6-Year, 81-Patient, Prospective Multicenter Study. Plast Reconstr Surg. 2012 May;129(5):1173–87. doi:10.1097/PRS.0b013e31824a2db6

92. Oranges CM, Striebel J, Tremp M, Madduri S, Kalbermatten DF, Schaefer DJ. The Impact of Recipient Site External Expansion in Fat Grafting Surgical Outcomes. Plast Reconstr Surg - Glob Open. 2018 Feb;6(2):e1649. doi:10.1097/GOX.0000000000001649

93. Koh YJ, Koh BI, Kim H, Joo HJ, Jin HK, Jeon J, et al. Stromal Vascular Fraction From Adipose Tissue Forms Profound Vascular Network Through the Dynamic Reassembly of Blood Endothelial Cells. Arterioscler Thromb Vasc Biol. 2011 May;31(5):1141–50. doi:10.1161/ATVBAHA.110.218206

94. Topcu A, Aydin OE, Ünlü M, Barutcu A, Atabey A. Increasing the Viability of Fat Grafts by Vascular Endothelial Growth Factor. Arch Facial Plast Surg. 2012 Jul 1;14(4). doi:10.1001/archfacial.2011.1633

95. Elçin YM, Dixit V, Gitnick G. Extensive In Vivo Angiogenesis Following Controlled Release of Human Vascular Endothelial Cell Growth Factor: Implications for Tissue Engineering and Wound Healing. Artif Organs. 2001 Jul;25(7):558–65. doi:10.1046/j.1525-1594.2001.025007558.x

96. Shoshani O, Livne E, Armoni M, Shupak A, Berger J, Ramon Y, et al. The Effect of Interleukin-8 on the Viability of Injected Adipose Tissue in Nude Mice: Plast Reconstr Surg. 2005 Mar;115(3):853–9. doi:10.1097/01.PRS.0000153036.71928.30

97. Farooqui W, Pommergaard HC, Rasmussen A. Remote ischemic preconditioning of transplant recipients to reduce graft ischemia and reperfusion injuries: A systematic review. Transplant Rev. 2018 Jan;32(1):10–5. doi:10.1016/j.trre.2017.06.001

98. Gassman AA, Lewis MS, Lee JC. Remote Ischemic Preconditioning Recipient Tissues Improves the Viability of Murine Fat Transfer. Plast Reconstr Surg. 2016 Jul;138(1):55e–63e. doi:10.1097/PRS.0000000000002295

99. Samdal F, Skolleborg KChr, Berthelsen B. The Effect of Preoperative Needle Abrasion of the Recipient Site on Survival of Autologous Free Fat Grafts in Rats. Scand J Plast Reconstr Surg Hand Surg. 1992 Jan;26(1):33–6. doi:10.3109/02844319209035180

100. Sezgin B, Ozmen S, Bulam H, Omeroglu S, Yuksel S, Cayci B, et al. Improving fat graft survival through preconditioning of the recipient site with microneedling. J Plast Reconstr Aesthet Surg. 2014 May;67(5):712–20. doi:10.1016/j.bjps.2014.01.019

101. Baran CN, Çelebioglu S, Sensöz Ö, Ulusoy G, Civelek B, Ortak T. The Behavior of Fat Grafts in Recipient Areas with Enhanced Vascularity. Plast Reconstr Surg. 2002 Apr 15;109(5):1646.

102. James IB, Bourne DA, DiBernardo G, Wang SS, Gusenoff JA, Marra K, et al. The Architecture of Fat Grafting II: Impact of Cannula Diameter. Plast Reconstr Surg. 2018 Nov;142(5):1219–25. doi:10.1097/PRS.0000000000004837

103. Harris WM, Plastini M, Kappy N, Ortiz T, Chang S, Brown S, et al. Endothelial Differentiated Adipose-Derived Stem Cells Improvement of Survival and Neovascularization in Fat Transplantation. Aesthet Surg J. 2019 Jan 17;39(2):220–32. doi:10.1093/asj/sjy130

104. Silverman KJ, Lund DP, Zetter BR, Lainey LL, Shahood JA, Freiman DG, et al. Angiogenic activity of adipose tissue. Biochem Biophys Res Commun. 1988 May;153(1):347–52. doi:10.1016/S0006-291X(88)81229-4

105. Xiong BJ, Tan QW, Chen YJ, Zhang Y, Zhang D, Tang SL, et al. The Effects of Platelet-Rich Plasma and Adipose-Derived Stem Cells on Neovascularization and Fat Graft Survival. Aesthetic Plast Surg. 2018 Feb;42(1):1–8. doi:10.1007/s00266-017-1062-1

106. Lee JH, Kirkham JC, McCormack MC, Nicholls AM, Randolph MA, Austen WG. The Effect of Pressure and Shear on Autologous Fat Grafting: Plast Reconstr Surg. 2013 May;131(5):1125–36. doi:10.1097/PRS.0b013e3182879f4a

107. Nguyen A, Pasyk KA, Bouvier TN, Hassett CA, Argenta LC. Comparative study of survival of autologous adipose tissue taken and transplanted by different techniques. Plast Reconstr Surg. 1990 Mar;85(3):378–86; discussion 387-389. PubMed PMID: 2304989.

108. Rieck B, Schlaak S. In Vivo Tracking of Rat Preadipocytes After Autologous Transplantation: Ann Plast Surg. 2003 Sep;51(3):294–300. doi:10.1097/01.SAP.0000063758.16488.A9

109. Khouri RK, Rigotti G, Cardoso E, Khouri RK, Biggs TM. Megavolume Autologous Fat Transfer: Part I. Theory and Principles. Plast Reconstr Surg. 2014 Mar;133(3):550–7. doi:10.1097/01.prs.0000438044.06387.2a

110. Parrish JN, Metzinger SE. Autogenous Fat Grafting and Breast Augmentation: A Review of the Literature. Aesthet Surg J. 2010 Jul;30(4):549–56. doi:10.1177/1090820X10380859

111. Fournier PF. Fat Grafting: My Technique.

112. Ersek RA. Transplantation of purified autologous fat: a 3-year follow-up is disappointing [Internet]. 1991 Feb. Available from: https://pubmed.ncbi.nlm.nih.gov/1750860/

113. Delay E, Garson S, Tousson G, Sinna R. Fat Injection to the Breast: Technique, Results, and Indications Based on 880 Procedures Over 10 Years. Aesthet Surg J. 2009 Sep;29(5):360–76. doi:10.1016/j.asj.2009.08.010

114. Nishimura T, Hashimoto H, Nakanishi I, Furukawa M. Microvascular Angiogenesis and Apoptosis in the Survival of Free Fat Grafts. The Laryngoscope. 2000 Aug;110(8):1333–8. doi:10.1097/00005537-200008000-00021

115. Gontijo-de-Amorim NF, Charles-de-Sá L, Rigotti G. Mechanical Supplementation With the Stromal Vascular Fraction Yields Improved Volume Retention in Facial Lipotransfer: A 1-Year Comparative Study. Aesthet Surg J. 2017 Oct 1;37(9):975–85. doi:10.1093/asj/sjx115

116. Kølle SFT, Fischer-Nielsen A, Mathiasen AB, Elberg JJ, Oliveri RS, Glovinski PV, et al. Enrichment of autologous fat grafts with ex-vivo expanded adipose tissue-derived stem cells for graft survival: a randomised placebo-controlled trial. The Lancet. 2013 Sep;382(9898):1113–20. doi:10.1016/S0140-6736(13)61410-5

117. Choi M, Small K, Levovitz C, Lee C, Fadl A, Karp NS. The Volumetric Analysis of Fat Graft Survival in Breast Reconstruction: Plast Reconstr Surg. 2013 Feb;131(2):185–91. doi:10.1097/PRS.0b013e3182789b13

118. Garza RM, Paik KJ, Chung MT, Duscher D, Gurtner GC, Longaker MT, et al. Studies in Fat Grafting: Part III. Fat Grafting Irradiated Tissue—Improved Skin Quality and Decreased Fat Graft Retention. Plast Reconstr Surg. 2014 Aug;134(2):249–57. doi:10.1097/PRS.0000000000000326

119. Gerth DJ, King B, Rabach L, Glasgold RA, Glasgold MJ. Long-Term Volumetric Retention of Autologous Fat Grafting Processed With Closed-Membrane Filtration. Aesthet Surg J. 2014 Sep 1;34(7):985–94. doi:10.1177/1090820X14542649

120. Herly M, Ørholt M, Glovinski PV, Pipper CB, Broholm H, Poulsgaard L, et al. Quantifying Long-Term Retention of Excised Fat Grafts: A Longitudinal, Retrospective Cohort Study of 108 Patients Followed for Up to 8.4 Years. Plast Reconstr Surg. 2017 May;139(5):1223–32. doi:10.1097/PRS.0000000000003237

121. Paik KJ, Zielins ER, Atashroo DA, Maan ZN, Duscher D, Luan A, et al. Studies in Fat Grafting: Part V. Cell-Assisted Lipotransfer to Enhance Fat Graft Retention Is Dose Dependent. Plast Reconstr Surg. 2015 Jul;136(1):67–75. doi:10.1097/PRS.0000000000001367

122. Varghese J, Griffin M, Mosahebi A, Butler P. Systematic review of patient factors affecting adipose stem cell viability and function: implications for regenerative therapy. Stem Cell Res Ther. 2017 Dec;8(1):45. doi:10.1186/s13287-017-0483-8

123. Zuk PA, Zhu M, Ashjian P, Ugarte DAD, Huang JI, Mizuno H, et al. Human Adipose Tissue Is a Source of Multipotent Stem Cells.

124. Matsumoto D, Sato K, Gonda K, Takaki Y, Shigeura T, Sato T, et al. Cell-Assisted Lipotransfer: Supportive Use of Human Adipose-Derived Cells for Soft Tissue Augmentation with Lipoinjection.

125. Yoshimura K, Sato K, Aoi N, Kurita M, Hirohi T, Harii K. Cell-Assisted Lipotransfer for Cosmetic Breast Augmentation: Supportive Use of Adipose-Derived Stem/Stromal Cells. Aesthetic Plast Surg. 2008 Jan;32(1):48–55. doi:10.1007/s00266-007-9019-4

126. Li FW, Wang HB, Fang JP, Zeng L, Chen CL, Luo SK. Optimal Use Ratio of the Stromal Vascular Fraction (SVF): An Animal Experiment Based on Micro-CT Dynamic Detection After Large-Volume Fat Grafting. Aesthet Surg J. 2019 May 16;39(6):NP213–24. doi:10.1093/asj/sjy266

127. Ni Y, He X, Yuan Z, Liu M, Du H, Zhong X. Effect of Fat Particle-to-SVF Ratio on Graft Survival Rates in Rabbits. Ann Plast Surg. 2015 May;74(5):609–14. doi:10.1097/SAP.0b013e318298e6f5

128. Kakudo N, Morimoto N, Ogawa T, Hihara M, Lai F, Kusumoto K. Adipose-derived stem cell (ASC)-enriched fat grafting: experiments using White rabbits and an automated cell processing apparatus. Med Mol Morphol. 2017 Sep;50(3):170–7. doi:10.1007/s00795-017-0158-7

129. Rasmussen BS, Sørensen CL, Kurbegovic S, Ørholt M, Talman MLM, Herly M, et al. Cell-Enriched Fat Grafting Improves Graft Retention in a Porcine Model: A Dose-Response Study of Adipose-Derived Stem Cells versus Stromal Vascular Fraction. Plast Reconstr Surg. 2019 Sep;144(3):397e–408e. doi:10.1097/PRS.0000000000005920

130. Choi JW, Kim SC, Park EJ, Lee JA, Jeong WS. Positive Effect of Incubated Adipose-Derived Mesenchymal Stem Cells on Microfat Graft Survival. J Craniofac Surg. 2018 Jan;29(1):243–7. doi:10.1097/SCS.0000000000004071

131. Zhu M, Xue J, Lu S, Yuan Y, Liao Y, Qiu J, et al. Anti-inflammatory effect of stromal vascular fraction cells in fat transplantation. Exp Ther Med. 2018 Dec 11. doi:10.3892/etm.2018.7082

132. Peltoniemi HH, Salmi A, Miettinen S, Mannerström B, Saariniemi K, Mikkonen R, et al. Stem cell enrichment does not warrant a higher graft survival in lipofilling of the breast: A prospective comparative study. J Plast Reconstr Aesthet Surg. 2013 Nov;66(11):1494–503. doi:10.1016/j.bjps.2013.06.002

133. Wang L, Luo X, Lu Y, Fan ZH, Hu X. Is the Resorption of Grafted Fat Reduced in Cell-Assisted Lipotransfer for Breast Augmentation? Ann Plast Surg. 2015 Aug;75(2):128–34. doi:10.1097/SAP.0000000000000068

134. Gentile P, Orlandi A, Scioli MG, Di Pasquali C, Bocchini I, Curcio CB, et al. A Comparative Translational Study: The Combined Use of Enhanced Stromal Vascular Fraction and Platelet-Rich Plasma Improves Fat Grafting Maintenance in Breast Reconstruction. Stem Cells Transl Med. 2012 Apr 1;1(4):341–51. doi:10.5966/sctm.2011-0065

135. Philips BJ, Marra KG, Rubin JP. Adipose stem cell-based soft tissue regeneration. Expert Opin Biol Ther. 2012 Feb;12(2):155–63. doi:10.1517/14712598.2012.644533

136. Fraser JK, Wulur I, Alfonso Z, Hedrick MH. Fat tissue: an underappreciated source of stem cells for biotechnology. Trends Biotechnol. 2006 Apr;24(4):150–4. doi:10.1016/j.tibtech.2006.01.010

137. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, et al. Multilineage Cells from Human Adipose Tissue: Implications for Cell-Based Therapies. Tissue Eng. 2001 Apr;7(2):211–28. doi:10.1089/107632701300062859

138. Sterodimas A, De Faria J, Nicaretta B, Pitanguy I. Tissue engineering with adipose-derived stem cells (ADSCs): Current and future applications. J Plast Reconstr Aesthet Surg. 2010 Nov;63(11):1886–92. doi:10.1016/j.bjps.2009.10.028

139. Baer PC. Adipose-Derived Stem Cells and Their Potential to Differentiate into the Epithelial Lineage. Stem Cells Dev. 2011 Oct;20(10):1805–16. doi:10.1089/scd.2011.0086

140. Ferroni L, Gardin C, Tocco I, Epis R, Casadei A, Vindigni V, et al. Potential for Neural Differentiation of Mesenchymal Stem Cells. Weyand B, Dominici M, Hass R, Jacobs R, Kasper C, editors. Mesenchymal Stem Cells - Basics Clin Appl I. 2012;Advances in Biochemical Engineering/Biotechnology129:89–115. doi:10.1007/10_2012_152

141. Moseley TA, Zhu M, Hedrick MH. Adipose-Derived Stem and Progenitor Cells as Fillers in Plastic and Reconstructive Surgery: Plast Reconstr Surg. 2006 Sep;118(Suppl):121S-128S. doi:10.1097/01.prs.0000234609.74811.2e

142. Miranville A, Heeschen C, Sengenès C, Curat CA, Busse R, Bouloumié A. Improvement of Postnatal Neovascularization by Human Adipose Tissue-Derived Stem Cells. Circulation. 2004 Jul 20;110(3):349–55. doi:10.1161/01.CIR.0000135466.16823.D0

143. Li M, Chen C. The Efficacy of Cell-Assisted Lipotransfer Versus Conventional Lipotransfer in Breast Augmentation: A Systematic Review and Meta-Analysis. Aesthetic Plast Surg. 2021 Aug;45(4):1478–86. doi:10.1007/s00266-020-02123-0

144. Hu Y, Wang X, Jiang C. Clinical Efficacy Analysis of Augmentation Mammoplasty with Cell-Assisted Autologous Fat Transplantation. Aesthetic Plast Surg. 2022 Oct;46(5):2218–27. doi:10.1007/s00266-022-02778-x

145. Toyserkani NM, Quaade ML, Sørensen JA. Cell-Assisted Lipotransfer: A Systematic Review of Its Efficacy. Aesthetic Plast Surg. 2016 Apr;40(2):309–18. doi:10.1007/s00266-016-0613-1

146. Kapur SK, Katz AJ. Review of the adipose derived stem cell secretome. Biochimie. 2013 Dec;95(12):2222–8. doi:10.1016/j.biochi.2013.06.001

147. Rehman J, Traktuev D, Li J, Merfeld-Clauss S, Temm-Grove CJ, Bovenkerk JE, et al. Secretion of Angiogenic and Antiapoptotic Factors by Human Adipose Stromal Cells. Circulation. 2004 Mar 16;109(10):1292–8. doi:10.1161/01.CIR.0000121425.42966.F1

148. Cai L, Johnstone BH, Cook TG, Liang Z, Traktuev D, Cornetta K, et al. Suppression of Hepatocyte Growth Factor Production Impairs the Ability of Adipose-Derived Stem Cells to Promote Ischemic Tissue Revascularization. Stem Cells. 2007 Dec 1;25(12):3234–43. doi:10.1634/stemcells.2007-0388

149. Kim WS, Park BS, Sung JH. The wound-healing and antioxidant effects of adipose-derived stem cells. Expert Opin Biol Ther. 2009 Jul;9(7):879–87. doi:10.1517/14712590903039684

150. Planat-Benard V, Silvestre JS, Cousin B, André M, Nibbelink M, Tamarat R, et al. Plasticity of Human Adipose Lineage Cells Toward Endothelial Cells: Physiological and Therapeutic Perspectives. Circulation. 2004 Feb 10;109(5):656–63. doi:10.1161/01.CIR.0000114522.38265.61

151. Verseijden F, Posthumus-van Sluijs SJ, Pavljasevic P, Hofer SOP, Van Osch GJVM, Farrell E. Adult Human Bone Marrow– and Adipose Tissue–Derived Stromal Cells Support the Formation of Prevascular-like Structures from Endothelial Cells *In Vitro*. Tissue Eng Part A. 2010 Jan;16(1):101–14. doi:10.1089/ten.tea.2009.0106

152. Tiryaki T, Findikli N, Tiryaki D. Staged Stem Cell-enriched Tissue (SET) Injections for Soft Tissue Augmentation in Hostile Recipient Areas: A Preliminary Report. Aesthetic Plast Surg. 2011 Dec;35(6):965–71. doi:10.1007/s00266-011-9716-x

153. Hsu VM, Stransky CA, Bucky LP, Percec I. Fat Grafting's Past, Present, and Future: Why Adipose Tissue Is Emerging as a Critical Link to the Advancement of Regenerative Medicine. Aesthet Surg J. 2012 Sep;32(7):892–9. doi:10.1177/1090820X12455658

154. Yoshimura K, Suga H, Eto H. Adipose-Derived Stem/Progenitor Cells: Roles in Adipose Tissue Remodeling and Potential Use for Soft Tissue Augmentation. Regen Med. 2009 Mar;4(2):265–73. doi:10.2217/17460751.4.2.265

155. Tissiani LAL, Alonso N. A Prospective and Controlled Clinical Trial on Stromal Vascular Fraction Enriched Fat Grafts in Secondary Breast Reconstruction. Ferraro GA, editor. Stem Cells Int. 2016 Jan;2016(1):2636454. doi:10.1155/2016/2636454

156. Chiu CH. Does Stromal Vascular Fraction Ensure a Higher Survival in Autologous Fat Grafting for Breast Augmentation? A Volumetric Study Using 3-Dimensional Laser Scanning. Aesthet Surg J. 2019 Jan 1;39(1):41–52. doi:10.1093/asj/sjy030

157. Zhou Y, Wang J, Li H, Liang X, Bae J, Huang X, et al. Efficacy and Safety of Cell-Assisted Lipotransfer: A Systematic Review and Meta-Analysis. Plast Reconstr Surg. 2016 Jan;137(1):44e–57e. doi:10.1097/PRS.0000000000001981

158. Vester-Glowinski PV, Herly M, Ørholt M, Rasmussen BS, Müller FC, Elberg JJ, et al. Fat Grafting With Expanded Adipose-Derived Stromal Cells for Breast Augmentation: A Randomized Controlled Trial. Aesthet Surg J. 2022 Oct 13;42(11):1279–89. doi:10.1093/asj/sjac159

159. Wang C, Long X, Si L, Chen B, Zhang Y, Sun T, et al. A Pilot Study On Ex Vivo Expanded Autologous Adipose-Derived Stem Cells of Improving Fat Retention in Localized Scleroderma Patients. Stem Cells Transl Med. 2021 Aug 1;10(8):1148–56. doi:10.1002/sctm.20-0419

160. Yin Y, Li J, Li Q, Zhang A, Jin P. Autologous fat graft assisted by stromal vascular fraction improves facial skin quality: A randomized controlled trial. J Plast Reconstr Aesthet Surg. 2020 Jun;73(6):1166–73. doi:10.1016/j.bjps.2019.11.010

161. Kølle SFT, Duscher D, Taudorf M, Fischer-Nielsen A, Svalgaard JD, Munthe-Fog L, et al. Ex vivo-expanded autologous adipose tissue-derived stromal cells ensure enhanced fat graft retention in breast augmentation: A randomized controlled clinical trial. Stem Cells Transl Med. 2020 Nov 1;9(11):1277–86. doi:10.1002/sctm.20-0081

162. Roshdy OH, Abdallah WI, Farid CI, Mehanna RA, Bayoumi NH, Ismail AI. Stromal vascular fraction improves the durability of autologous fat temple augmentation—A split-face randomized study using ultrasound biomicroscopy. J Plast Reconstr Aesthet Surg. 2022 Jun;75(6):1870–7. doi:10.1016/j.bjps.2021.12.005

163. Chen A, Zhang L, Chen P, Zhang C, Tang S, Chen X. Comparison of the Efficacy and Safety of Cell-Assisted Lipotransfer and Platelet-Rich Plasma Assisted Lipotransfer: What Should We Expect from a Systematic Review with Meta-Analysis? Cell Transplant. 2021 Jan 1;30:0963689721989607. doi:10.1177/0963689721989607

164. Gentile P, De Angelis B, Pasin M, Cervelli G, Curcio CB, Floris M, et al. Adipose-Derived Stromal Vascular Fraction Cells and Platelet-Rich Plasma: Basic and Clinical Evaluation for Cell-Based Therapies in Patients With Scars on the Face. J Craniofac Surg. 2014 Jan;25(1):267–72. doi:10.1097/01.scs.0000436746.21031.ba

165. Jeon HJ, Choi DH, Lee JH, Lee JS, Lee J, Park HY, et al. A Prospective Study of the Efficacy of Cell-Assisted Lipotransfer with Stromal Vascular Fraction to Correct Contour Deformities of the Autologous Reconstructed Breast. Aesthetic Plast Surg. 2021 Jun;45(3):853–63. doi:10.1007/s00266-020-01981-y

166. Bourne DA, Egro FM, Bliley J, James I, Haas GL, Meyer EM, et al. Stem Cell Therapy Enriched Fat Grafting for the Reconstruction of Craniofacial Deficits. Plast Reconstr Surg - Glob Open. 2023 Jun;11(6):e5056. doi:10.1097/GOX.0000000000005056

167. Wufuer M, Choi TH, Najmiddinov B, Kim J, Choi J, Kim T, et al. Improving Facial Fat Graft Survival Using Stromal Vascular Fraction–Enriched Lipotransfer: A Multicenter Randomized Controlled Study. Plast Reconstr Surg. 2024 Apr;153(4):690e–700e. doi:10.1097/PRS.0000000000010625

168. Mizuno H, Hyakusoku H. Fat Grafting to the Breast and Adipose-Derived Stem Cells: Recent Scientific Consensus and Controversy. Aesthet Surg J. 2010 May 1;30(3):381–7. doi:10.1177/1090820X10373063

169. Jung HK, Kim CH, Song SY. Prospective 1-Year Follow-Up Study of Breast Augmentation by Cell-Assisted Lipotransfer. Aesthet Surg J. 2016 Feb;36(2):179–90. doi:10.1093/asj/sjv164

170. Fontdevila J, Guisantes E, Martínez E, Prades E, Berenguer J. Double-Blind Clinical Trial to Compare Autologous Fat Grafts versus Autologous Fat Grafts with PDGF: No Effect of PDGF. Plast Reconstr Surg. 2014 Aug;134(2):219e–30e. doi:10.1097/PRS.0000000000000409

171. Gentile P, Piccinno MS, Calabrese C. Characteristics and Potentiality of Human Adipose-Derived Stem Cells (hASCs) Obtained from Enzymatic Digestion of Fat Graft. Cells. 2019 Mar 25;8(3):282. doi:10.3390/cells8030282

172. Eto H, Kato H, Suga H, Aoi N, Doi K, Kuno S, et al. The Fate of Adipocytes after Nonvascularized Fat Grafting: Evidence of Early Death and Replacement of Adipocytes. Plast Reconstr Surg. 2012 May;129(5):1081–92. doi:10.1097/PRS.0b013e31824a2b19

173. Kato H, Mineda K, Eto H, Doi K, Kuno S, Kinoshita K, et al. Degeneration, Regeneration, and Cicatrization after Fat Grafting: Dynamic Total Tissue Remodeling during the First 3 Months. Plast Reconstr Surg. 2014 Mar;133(3):303e–13e. doi:10.1097/PRS.0000000000000066

174. Dos Anjos S, Matas-Palau A, Mercader J, Katz AJ, Llull R. Reproducible Volume Restoration and Efficient Long-term Volume Retention after Point-of-care Standardized Cell-enhanced Fat Grafting in Breast Surgery: Plast Reconstr Surg - Glob Open. 2015 Oct;3(10):e547. doi:10.1097/GOX.0000000000000511

175. Mashiko T, Wu SH, Feng J, Kanayama K, Kinoshita K, Sunaga A, et al. Mechanical Micronization of Lipoaspirates: Squeeze and Emulsification Techniques. Plast Reconstr Surg. 2017 Jan;139(1):79–90. doi:10.1097/PRS.0000000000002920

176. Gentile P, Kothari A, Casella D, Calabrese C. Fat Graft Enhanced With Adipose-Derived Stem Cells in Aesthetic Breast Augmentation: Clinical, Histological, and Instrumental Evaluation. Aesthet Surg J. 2020 Aug 14;40(9):962–77. doi:10.1093/asj/sjz292

177. Laloze J, Varin A, Bertheuil N, Grolleau JL, Vaysse C, Chaput B. Cell-assisted lipotransfer: Current concepts. Ann Chir Plast Esthét. 2017 Dec;62(6):609–16. doi:10.1016/j.anplas.2017.03.011

178. Jotzu C, Alt E, Welte G, Li J, Hennessy BT, Devarajan E, et al. Adipose Tissue-Derived Stem Cells Differentiate into Carcinoma-Associated Fibroblast-Like Cells under the Influence of Tumor-Derived Factors. Anal Cell Pathol. 2010;33(2):61–79. doi:10.1155/2010/695162

179. Yu JM, Jun ES, Bae YC, Jung JS. Mesenchymal Stem Cells Derived from Human Adipose Tissues Favor Tumor Cell Growth *in vivo*. Stem Cells Dev. 2008 Jun;17(3):463–74. doi:10.1089/scd.2007.0181

180. Zhao M, Dumur CI, Holt SE, Beckman MJ, Elmore LW. Multipotent adipose stromal cells and breast cancer development: Think globally, act locally. Mol Carcinog. 2010 Nov;49(11):923–7. doi:10.1002/mc.20675

181. Zimmerlin L, Donnenberg AD, Rubin JP, Basse P, Landreneau RJ, Donnenberg VS. Regenerative Therapy and Cancer: *In Vitro* and *In Vivo* Studies of the Interaction Between Adipose-Derived Stem Cells and Breast Cancer Cells from Clinical Isolates. Tissue Eng Part A. 2011 Jan;17(1–2):93–106. doi:10.1089/ten.tea.2010.0248

182. Claro F, Figueiredo JCA, Zampar AG, Pinto-Neto AM. Applicability and safety of autologous fat for reconstruction of the breast. Br J Surg. 2012 Apr 27;99(6):768–80. doi:10.1002/bjs.8722

183. Largo RD, Tchang LAH, Mele V, Scherberich A, Harder Y, Wettstein R, et al. Efficacy, safety and complications of autologous fat grafting to healthy breast tissue: A systematic review. J Plast Reconstr Aesthet Surg. 2014 Apr;67(4):437–48. doi:10.1016/j.bjps.2013.11.011

184. Donnenberg VS, Zimmerlin L, Rubin JP, Donnenberg AD. Regenerative Therapy After Cancer: What Are the Risks? Tissue Eng Part B Rev. 2010 Dec;16(6):567–75. doi:10.1089/ten.teb.2010.0352

185. Frese L, Dijkman PE, Hoerstrup SP. Adipose Tissue-Derived Stem Cells in Regenerative Medicine. Transfus Med Hemotherapy. 2016;43(4):268–74. doi:10.1159/000448180

186. Raposio E, Ciliberti R. Clinical use of adipose-derived stem cells: European legislative issues. Ann Med Surg. 2017 Dec;24:61–4. doi:10.1016/j.amsu.2017.11.002

187. Grabin S, Antes G, Stark GB, Motschall E, Buroh S, Lampert FM. Cell-Assisted Lipotransfer. Dtsch Ärztebl Int. 2015 Apr 10. doi:10.3238/arztebl.2015.0255

188. Simonacci F, Bertozzi N, Raposio E. Off-label use of adipose-derived stem cells. Ann Med Surg. 2017 Dec;24:44–51. doi:10.1016/j.amsu.2017.10.023

10 Attachment

10.1 PRISMA 2020 Checklist

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	fulfilled
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	fulfilled
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	4.4
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	4.5; 4.6
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	5.2; 5.6
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	5.1
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	5.1
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	5.3
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	5.3; 5.4; 5.5
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	5.4; 5.6
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	5.4; 5.6
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	5.5
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	5.6
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	5.2; 5.6; 6.1
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	5.6; 6.2.2
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	5.6; 6.1
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	5.6
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	5.6
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	/
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	5.5
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	5.5

Section and Topic	Item #	Checklist item	Location where item is reported
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	5.3; Figure1; 6.1
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	/
Study characteristics	17	Cite each included study and present its characteristics.	6.1; Table1
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	5.5; 6.1; Table1
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	6.2-3; Table1; Figure2-3
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	6.1-2
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	6.2-3
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	6.2; 6.4-6
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	/
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	5.5; 7.6
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	5.5
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	7.1-5; 8
	23b	Discuss any limitations of the evidence included in the review.	7.6
	23c	Discuss any limitations of the review processes used.	7.6
	23d	Discuss implications of the results for practice, policy, and future research.	7.7-8; 8
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	7.6
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	7.6
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	/
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	/
Competing interests	26	Declare any competing interests of review authors.	/
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	/