



Enrichment of autologous fat grafts with ex-vivo expanded adipose tissue-derived stem cells for graft survival: a randomised placebo-controlled trial

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Summary

Background Autologous fat grafting is increasingly used in reconstructive surgery. However, resorption rates ranging from 25% to 80% have been reported. Therefore, methods to increase graft viability are needed. Here, we report the results of a triple-blind, placebo-controlled trial to compare the survival of fat grafts enriched with autologous adipose-derived stem cells (ASCs) versus non-enriched fat grafts.

Methods Healthy participants underwent two liposuctions taken 14 days apart: one for ASC isolation and ex-vivo expansion, and another for the preparation of fat grafts. Two purified fat grafts (30 mL each) taken from the second liposuction were prepared for each participant. One graft was enriched with ASCs (20×10^6 cells per mL fat), and another graft without ASC enrichment served as a control. The fat grafts were injected subcutaneously as a bolus to the posterior part of the right and left upper arm according to the randomisation sequence. The volumes of injected fat grafts were measured by MRI immediately after injection and after 121 days before surgical removal. The primary goal was to compare the residual graft volumes of ASC-enriched grafts with those of control grafts. This study is registered at www.clinicaltrialsregister.eu, number 2010-023006-12.

Findings 13 participants were enrolled, three of whom were excluded. Compared with the control grafts, the ASC-enriched fat grafts had significantly higher residual volumes: $23 \cdot 00$ (95% CI $20 \cdot 57$ – $25 \cdot 43$) cm^3 versus $4 \cdot 66$ ($3 \cdot 16$ – $6 \cdot 16$) cm^3 for the controls, corresponding to 80·9% ($76 \cdot 6$ – $85 \cdot 2$) versus 16·3% ($11 \cdot 1$ – $21 \cdot 4$) of the initial volumes, respectively ($p < 0 \cdot 0001$). The difference between the groups was $18 \cdot 34$ (95% CI $15 \cdot 70$ – $20 \cdot 98$) cm^3 , equivalent to 64·6% ($57 \cdot 1$ – $72 \cdot 1$; $p < 0 \cdot 0001$). No serious adverse events were noted.

Interpretation The procedure of ASC-enriched fat grafting had excellent feasibility and safety. These promising results add significantly to the prospect of stem cell use in clinical settings, and indicate that ASC graft enrichment could render lipofilling a reliable alternative to major tissue augmentation, such as breast surgery, with allogeneic material or major flap surgery.

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Introduction

Autologous fat grafting is rapidly increasing in popularity worldwide. This procedure is used in breast reconstruction after cancer treatment; in the treatment of burn scars, congenital malformations, and post-traumatic malformations; for rejuvenation; and for the treatment of other conditions. Sustainable fillers are of the utmost importance in reconstructive surgery. Autologous fat is considered ideal for soft tissue augmentation because it is biocompatible, versatile, natural-looking, non-immunogenic, inexpensive, and readily obtainable, with low donor site morbidity.^{1,2} The major obstacle is an unpredictable and often low graft survival, with resorption rates ranging from 25% to 80%.^{3–7} Therefore, methods to increase graft viability are required.

Several studies have assessed the effects of cell-assisted lipotransfer.^{8,9} In this procedure, fat grafts are enriched with a freshly isolated stromal vascular fraction containing a mixed cell population that includes a minor fraction of adipose-derived stem cells

(ASCs).¹⁰ However, the reported results were not substantially better than those obtained with traditional lipofilling.^{8,9,11,12}

By contrast, recent animal studies have shown that fat grafts enriched with ex-vivo expanded ASCs markedly improved residual graft volume and histological appearance.^{13–15} We aimed to clarify whether ASC-enrichment of fat grafts in humans could improve graft survival and thereby make lipofilling procedures an attractive alternative to tissue augmentation with allogeneic material or major tissue flap surgery. In this study, we chose to use a bolus injection for the following reasons: to reflect the clinical characteristics of large-scale lipofilling, such as in breast reconstruction after mastectomy, where the recipient bed has very little well-vascularised tissue in which to place a large graft; to easily determine if the added ASCs had any effect in a setting in which unmanipulated fat grafts are known to have very poor survival; and to improve the ease of removal of fat grafts at the end of the study for proper histological evaluation

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and because the participants were healthy with no need for lipoinjections.

Methods

Study overview

This was a single-centre, triple-blind, randomised, placebo-controlled trial undertaken at Copenhagen University Hospital Rigshospitalet, Denmark. A flow-chart overview of the study is shown in figure 1. A follow-up period of 121 days was chosen because transplanted fat resorption is steady after roughly 3 months.^{9,12,16}

The study protocol complied with the Declaration of Helsinki and was approved by the Danish National Committee on Biomedical Research Ethics for Copenhagen, the Danish Data Protection Agency, and the Danish Medicines Agency. The project protocol is presented in the appendix. All patients provided written informed consent.

See Online for appendix

Patient population and randomisation

On the basis of the power calculations described below, we planned to include 13 participants. However, to include only the necessary number of participants, an interim analysis was done after the first ten patients had been assessed, as recommended by the Danish National Committee on Biomedical Research Ethics for the Capital Region of Denmark.

Inclusion criteria were: age 18–45 years; body-mass index 20–30 kg/m²; the participant requested abdominal liposuction and was considered suitable for liposuction from a surgical point of view, as assessed by the expected cosmetic results; and the participant provided signed informed consent. Exclusion criteria were: known chronic disease; smoking; previous abdominal surgery except

minor laparoscopic surgery; pregnancy or planned pregnancy within a year after the procedure; breastfeeding; allergy to the drugs used for general anaesthesia; and withdrawal of informed consent. Patients were excluded during the trial if there was microbiological contamination of either the cell culture or the transplanted tissue. All participants included were assessed by two experts in plastic surgery.

The posterior parts of both upper arms were chosen for fat grafting. One arm was implanted with an ASC-enriched fat graft and the other arm with a non-enriched control fat graft. The allocation sequence was generated using an online randomisation generator (www.randomization.com) and was concealed by a person unrelated to the trial management group and monitored by the Good Clinical Practice unit. The participants, study personnel, and outcome assessors were all blinded to treatment allocation, and blinding was maintained until all data had been analysed.

The primary outcome was residual volume based on MRI. The secondary outcomes were: the distribution of adipose tissue, connective tissue, newly formed connective tissue, and necrosis assessed by histological grafts examination; transplant vessel density assessed by histological examination of the grafts; and safety and feasibility.

Procedures

Fat tissue was harvested under local anaesthesia from only one side of the abdomen to leave the other side unmanipulated for the liposuction and graft preparation procedure. In total, 50 mL of fat tissue was harvested by standard sterile liposuction techniques as described by Coleman.^{11,17}

ASCs were isolated and culture-expanded as previously described¹⁸ using good manufacturing practice (GMP)-grade reagents in a certified laboratory approved by the Danish National Board of Health for clinical stem cell expansion. Stromal vascular fraction was isolated by collagenase (NB 6 GMP grade, SERVA Electrophoresis GmbH, Heidelberg, Germany) digestion and seeded in ten 1000 cm² flasks (Nunc A/S, Roskilde, Denmark) at a density of 5000 cells per cm² and four 75 cm² control flasks at a density of 4.0×10^5 cells per cm². After 24 h, the non-adherent cells were discarded. The cells were expanded in culture medium consisting of Dulbecco's modified Eagle's medium (PAA Laboratories GmbH, Pasching, Austria), 1% penicillin-streptomycin (GIBCO-Invitrogen, Taastrup, Denmark), 2 IU/mL preservative-free heparin (LEO Pharma, Ballerup, Denmark), and 10% pooled human platelet lysate (pHPL) for 2 weeks. The fragment-depleted pHPL was prepared from buffy-coats and plasma from voluntary blood donations using a freeze-thaw procedure as described by Schallmoser and colleagues,¹⁹ with minor modifications.¹⁸ Media were changed every 3 days. After 7 days of incubation, and again on the day of final ASC harvesting, a

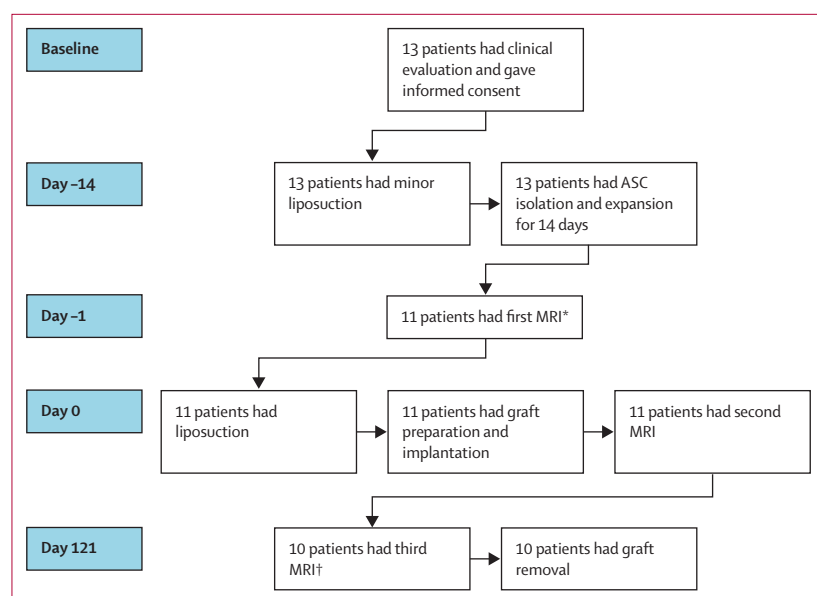


Figure 1: Study overview

*Two patients excluded because of ASC disposal due to contamination of one batch of growth medium.

†One patient excluded because of a minor complication in the surgical procedure.

supernatant medium sample was examined for microbiological contamination.

ASCs were harvested in the primary passage using TrypLE Select (Invitrogen, Taastrup, Denmark) to detach the cells. The release criteria included a negative microbiological sample 7 days before harvest, viability of more than 95%, and acceptable morphology. Phenotypic surface markers were also measured. After harvest, the ASC pellet (roughly 7.5 mL) was washed three times with phosphate-buffered saline (BioWhittaker, Walkersville, MD, USA) and resuspended in 1 mL saline with 1% human albumin (CSL Behring, Lyngby, Denmark). Cells were counted in three technical replicates using a NucleoCounter (Chemotec, Allerød, Denmark), and the average cell count was calculated. 6.5×10^8 ASCs were transferred to a 50 mL red Luer-Lok syringe (BD, Albertslund, Denmark). An identical empty syringe was prepared for placebo injection. We chose an ASC dose of 2000 times the physiological level based on the murine studies of Lu and colleagues¹³ and Zhu and colleagues,¹⁴ who used 1250 and 6000 times the physiological level, respectively. Additionally, we estimated that this amount would correspond to the largest cell dose obtainable in a clinical setting for large-scale lipofilling. Syringes were marked with the participant identification number and the injection side according to the allocation sequence by an independent person, thus maintaining the blinding of study personnel.

The abdominal liposuction and upper arm lipoinjection areas were marked with the participant in the upright position. Fat tissue was harvested from the side of the abdomen not previously used for fat harvest. The procedure was done under general anaesthesia following the principles described by Coleman.^{11,17}

After injecting a wetting solution, the fat tissue was harvested using a 3 mm diameter cannula with a blunt tip (Byron Medical, Wokingham, Berkshire, UK), connected to a 50 mL Luer-Lok syringe (BD, Albertslund, Denmark). After centrifugation at 3000 rpm for 3 min, the middle layer of the separated mixture, composed of predominantly fat tissue, was used for grafting. It was gently mixed in a bowl to evenly distribute the heterogeneous cell population. After syringe aspiration, classic liposuction was done to contour the body shape. A trained nurse transferred 34 mL of fat tissue to the control syringe and 30 mL to the syringe containing 4 mL of ASCs, resulting in an equal volume in both syringes. The syringes used were 50 mL red Luer-Lok (BD, Albertslund, Denmark). Both the ASC-enriched and non-enriched fat tissues were mixed six times using two 50 mL syringes with a Luer-to-Luer connection. The fat grafts were injected as a bolus, using a sharp 14-G cannula (MediPlast, Neumünster, Germany), subcutaneously 8 cm inferior to the anterior axillary fold and 3 cm posterior to the posterior edge of the medial bicipital groove. The graft location was marked by tattooing the skin with four small dots (appendix).

MRI was done at 3.0 Tesla (Siemens Magnetom Verio, Erlangen, Germany) with a four-channel flex coil. Markers were used to locate the injection site. Imaging was done with an axial 3D-VIBE sequence (TR/TE/TE 5.64 ms/2.45 ms/3.675 ms) with a thickness of 2 mm, 245×245 mm field of view, and 320×320 matrix size. Four T1-weighted images were calculated: in phase, out of phase, water only, and fat only. The arm was then imaged in the axial plane with a turbo STIR (short TI inversion recovery) sequence (TR/TE/TI/number of echoes 4520 ms/38 ms/220 ms/19) with a thickness of 2 mm, 245×245 mm field of view, and 256×256 matrix size.

Baseline images before transplantation, 30 min after transplantation, and just before removal were registered

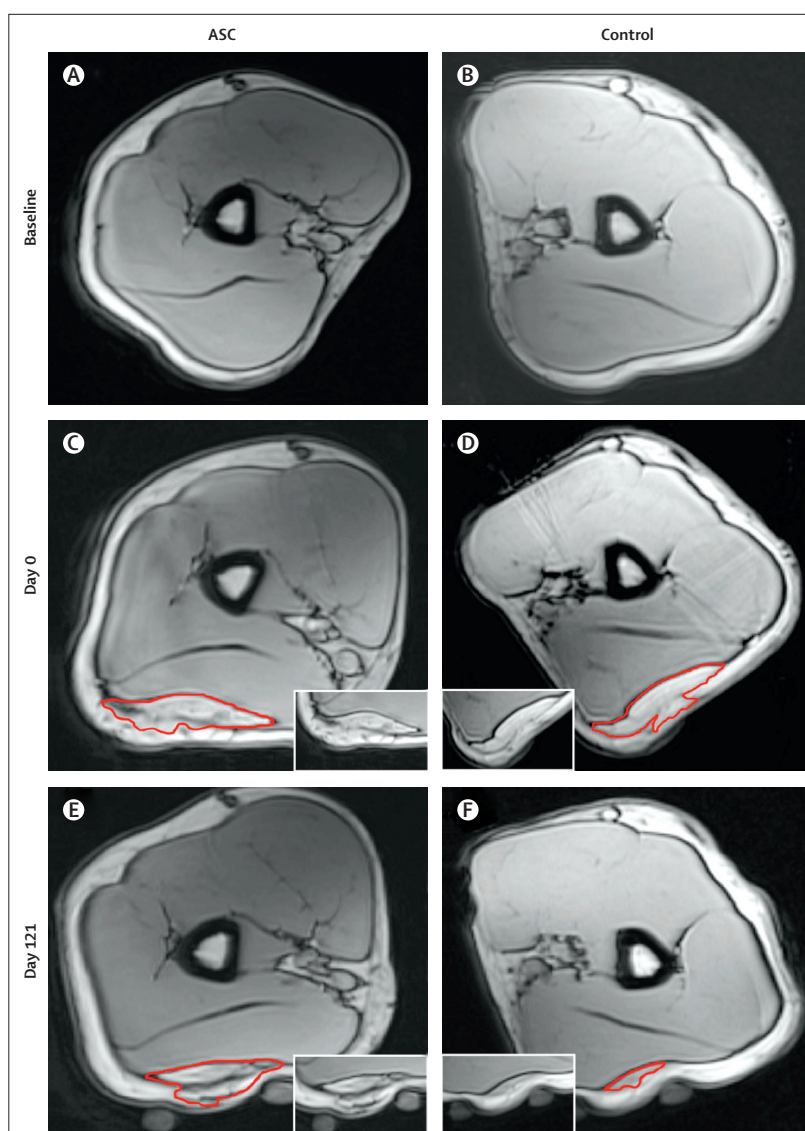


Figure 2: MR images and delineation of the implanted grafts

Baseline MR images of the target areas before implantation of (A) an ASC-enriched graft and (B) a control graft. (C, D) Post-implantation MR images of the target area with delineation of the graft. (E, F) The same areas on day 121 before graft removal. The inset images show the graft areas without delineation, for reference.

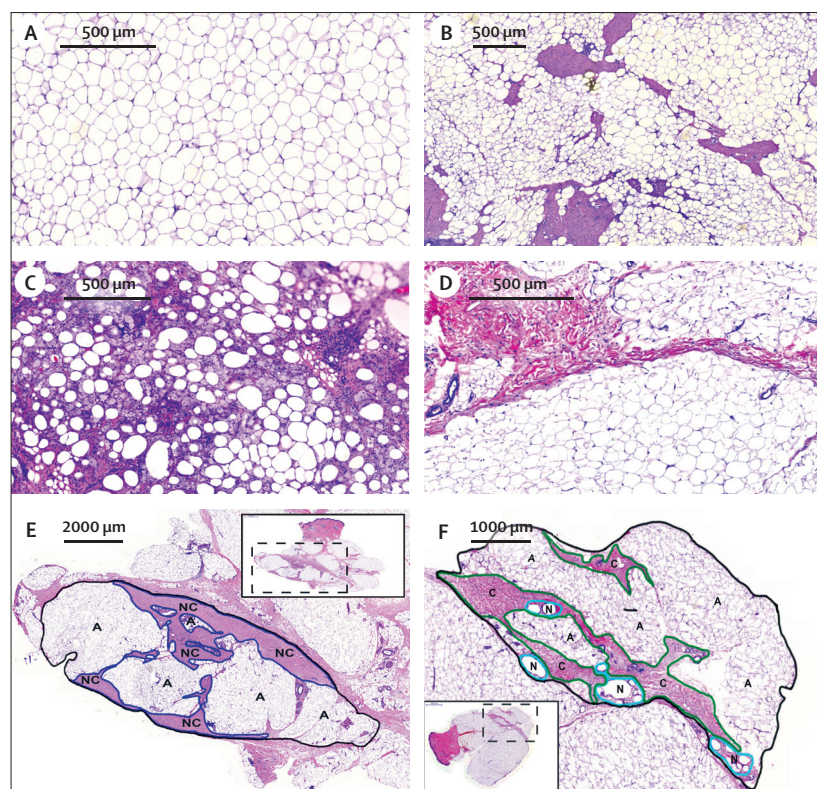


Figure 3: Tissue characteristics and delineation of the transplanted tissue

Representative reference tissue samples: (A) adipose tissue; (B) newly formed connective tissue; (C) necrotic tissue; and (D) connective tissue. Histological characterisation of the transplant and the different tissue types within the transplant of a representative sample. (E) A graft containing newly formed connective tissue and adipose tissue. (F) A graft containing normal connective tissue, necrotic areas, and adipose tissue. C=normal connective tissue. N=necrotic areas. A=adipose tissue. NC=newly formed connective tissue.

using anatomical landmarks in the subcutaneous fat. The transplants were identified on the STIR image and out-of-phase image. The fat transplant volumes were calculated by drawing one or more regions of interest around the transplanted tissue on the out-of-phase and STIR image. The area was multiplied by the slice thickness, giving the volume. This procedure was repeated for all slices (figure 2). On the day of transplant removal, the transplant locations were marked on the skin by identifying the transplant on the out-of-phase and STIR images with reference to the external markers. On day 121, the transplants and the tattooed markers were removed under local anaesthesia by dissection (appendix). The transplants or specimens were immediately examined histologically.

To determine the surface marker expression, ASC samples were harvested the day before the grafting procedure from the control flasks. We used a mesenchymal stem cell phenotyping kit (Miltenyi, Bergisch-Gladbach, Germany), which includes: a mixture of antibodies against mesenchymal stem cell-defining surface markers;²⁰ anti-CD73 (APC), anti-CD90 (FITC), and anti-CD105 (PE); antibody mixture against haemopoietic cell markers (CD14, CD20, CD34, and CD45-PerCP); and relevant isotype controls. We also analysed the expression of

HLA-DR using anti-HLA-DR PerCP (Miltenyi, Bergisch-Gladbach, Germany). All antibodies were used according to the manufacturer's instructions. The samples were analysed using a FACSCanto flow cytometer, and the data were analysed with FACSDiva software v.5.0.3 (Becton-Dickinson, Albertslund, Denmark).

Histology

The marked specimens were measured, photographed, and cut into 3-mm slices. The slices were formalin-fixed, paraffin-embedded, cut into 4-µm slices, mounted on glass slides, dehydrated, and stained with haematoxylin and eosin. The slides were scanned using a panoramic slide scanner (3D-Histech, Budapest, Hungary). The control evaluation was done with light microscopy (Olympus, Ballerup, Denmark).

The following areas were measured using Panoramic Viewer software (3D-Histech, Budapest, Hungary): total transplant, necrosis, newly formed connective tissue, morphologically normal adipose tissue; and connective tissue (figure 3). An area was included if changes not normally observed in subcutaneous tissue were seen.

Vessel density was characterised by using an Ultra-viewer visualisation system (Ventana Medical Systems, Tucson, AZ, USA) to count the number of vessels in defined transplant areas, using a polyclonal immuno-histochemical antibody against von Willebrand factor, diluted 1:15 000 (Dako, Glostrup, Denmark) according to the manufacturer's instructions. The results are reported as the vessel number/mm². The vessels were defined as elongated configurations or clearly visible lumina, leaving out all other stained areas.

Statistical analysis

All statistical analyses were done using SPSS 20 (IBM, Chicago, IL, USA), and graphs were generated using Prism 6 (GraphPad Software, La Jolla, CA, USA). A paired Student's *t* test was used for all comparisons. All data fulfilled the requirements for normality and equal variances.²¹ A two-sided *p*<0.05 was considered statistically significant.

We calculated that a sample size of 13 participants would be necessary to show a significant difference in residual graft volume of 25%, with an expected standard deviation of 20% using a study power of 90% and a significance level of 5%. The expected values were based on a murine model by Lu and colleagues.¹³ An interim analysis was done after the first ten patients had completed the study procedures and the appropriate data had been collected.

The study is registered at www.clinicaltrialsregister.eu (EudraCT number 2010-023006-12). The project was monitored by the local Good Clinical Practice unit (project number 2010-362).

Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or

writing of the report. All authors had full access to all the data after the allocation sequence code was broken. The corresponding author had final responsibility for the decision to submit for publication.

Results

13 healthy participants were enrolled (11 women and two men), three of whom were excluded. One woman (the first participant) was excluded because of a minor complication in the surgical procedure wherein the transplant was placed too close to the medial bicipital groove, resulting in a temporarily compromised venous back-flow. The transplant was removed, and the participant recovered completely. For the rest of the participants, the transplants were placed on the posterior part of the upper arm. One man and one woman were excluded due to ASC disposal during ex-vivo expansion because of contamination of one batch of growth medium. Thereafter, the manufacturing process was altered. All three participants are in good health and remain in our outpatient follow-up programme. The mean age of the remaining ten participants was 28.4 (95% CI 22.0–34.8) years. The mean body-mass index was 24.7 (95% CI 23.3–26.1).

The interim analysis revealed that the differences in the residual graft volumes were higher than expected. Therefore, there was no need to include additional participants to maintain 90% power in the final analysis. The recruitment of participants started in December 2011, and the follow-up period ended in September, 2012.

A cell pellet of around 7.5 mL was harvested from all ten 1000 cm² flasks containing 8.58 (95% CI 7.43–9.73) × 10⁸ ASCs. A mean of 28.20 (95% CI 25.94–30.46) mL of ASC-enriched transplant, and a mean of 28.60 (26.26–30.94) mL of the fat-alone transplant was injected, with a difference between groups of 0.40 (–0.10 to 0.90) mL (*p*=0.78).

Apart from the first included participant, there were no complications during the liposuction, lipofilling, and

transplant removal procedures. During the first 24 h post-transplantation, all participants reported mild redness and swelling at what seemed to be the site of the ASC-enriched fat graft. Otherwise, there were no adverse events.

On the MRI, the mean initial graft volumes were 28.38 (SD 3.20; 95% CI 26.10–30.67) cm³ for the ASC-enriched grafts and 28.84 (3.08; 26.63–31.04) cm³ for the control grafts. This corresponds well to the injected volumes. On day 121, the residual volumes were 23.00 (95% CI 20.57–25.43) cm³ and 4.66 (3.16–6.16) cm³ of ASC-enriched grafts and control grafts, respectively, corresponding to 80.9% (76.6–85.2) and 16.3% (11.1–21.4) of the initial volumes (*p*<0.0001). The difference between groups was 18.34 (95% CI 15.70–20.98) cm³, equivalent to 64.6% (57.1–72.1; *p*<0.0001; figure 4).

For all ex-vivo expanded cell lines used for fat grafting, more than 95% of the cells were positive for CD90 and CD73 expression, as defined from the isotype controls. In seven of ten participants, more than 95% of the cells were positive for CD105 expression, whereas in three participants the CD105-positive cells constituted 70–85%. Haemopoietic surface markers were weakly expressed on 2–4% of the cells of eight participants. For the cells of two participants, the results of duplicate assays varied considerably, with a percentage of roughly 2% in one assay and 24% in the other assay. Additionally, 2–6% of cells were positive for HLA-DR in all but one participant, in whom 12% of the cells expressed HLA-DR. The cells used for fat grafting were thus dominated by cells with a mesenchymal stem cell phenotype.

In the ASC-enriched grafts, there was a significantly higher amount of residual total adipose tissue compared with the control grafts: 84.3% (95% CI 80.0–88.6) versus 67.0% (55.2–78.8), respectively, with a difference of 17.3% (5.1–29.6; *p*=0.0109). A significantly higher amount of newly formed connective tissue was also found in the ASC-enriched grafts than in the control

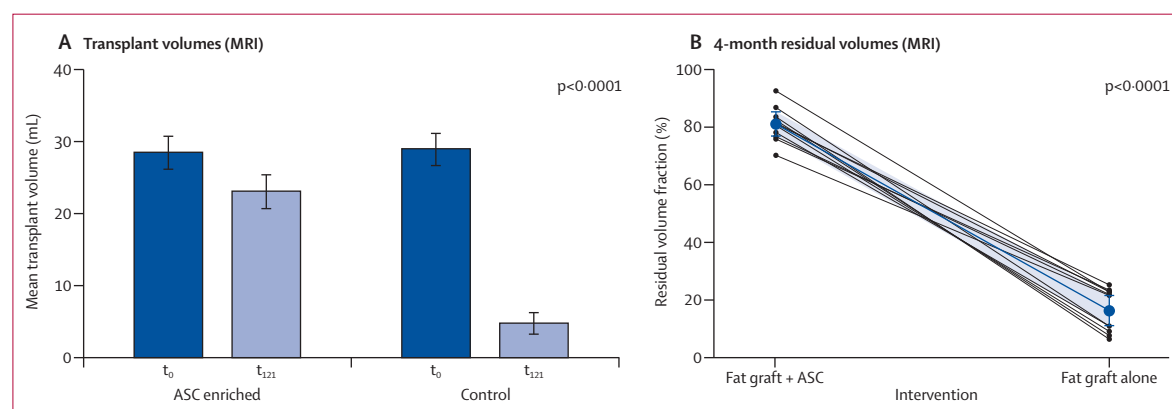


Figure 4: Transplant volumes

(A) Transplant volumes in ASC-enriched grafts and control grafts at day 0 and day 121. The plots show the means with 95% CIs. (B) Spaghetti plot of the residual transplant volume fractions in ASC-enriched grafts and control grafts. The mean residual volumes with 95% CIs are highlighted.

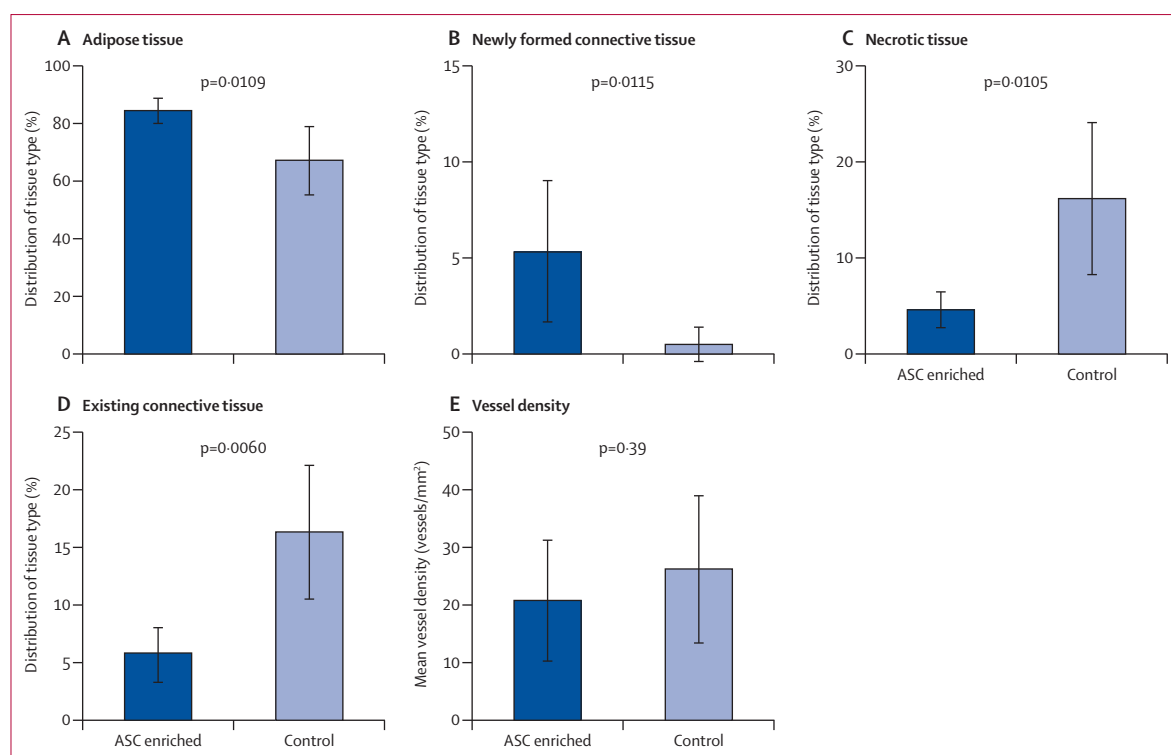


Figure 5: Histology

Distribution of the different types of tissues and vessel density in ASC-enriched grafts and control grafts. (A) Adipose tissue. (B) Newly formed connective tissue. (C) Necrotic tissue. (D) Connective tissue. (E) Vessel density. A–D: Percentage distribution and E: vessels/mm². The plots show the means with 95% CIs.

Panel: Research in context

Systematic review

We searched PubMed for original studies using the following search terms: (“mesenchymal stem cells” OR “mesenchymal stromal cells” OR “adipose stem cells” OR “processed lipoaspirate cells” OR “pre-adipocytes” OR “stromal vascular fraction stem cells” OR “adipose-derived stromal cells” OR “adipose tissue-derived stem cells”) AND (“cell-assisted lipotransfer” OR “lipofilling”). We assessed the quality of the evidence by critically reviewing the methods used in each study and by focusing on randomised controlled trials and randomised animal studies. We did not find any randomised clinical trials assessing the effect of ex-vivo expanded ASC-enriched fat grafts. Animal models showed a significant improvement in graft survival and the normal histological appearance of the transplanted tissue.^{13,14} We undertook a first-in-human blinded randomised controlled study to assess the effects of supplemented ex-vivo expanded ASCs on fat graft survival for large-scale reconstructive procedures.

Interpretation

We report a highly significant and substantial effect of added stem cells in this trial of ASC-enriched fat grafting. The procedure had excellent feasibility and safety. These promising results add significantly to the prospect of stem cell use in clinical settings and show that ASC graft enrichment could render lipofilling a reliable procedure, since the resorption rate, quality of tissue, and safety can be predicted. This procedure might prove to be a viable alternative to major tissue augmentation, such as breast augmentation, with allogeneic material or major tissue flap surgery with fewer side-effects, better safety, and more satisfying cosmetic results.

grafts: 5.3% (95% CI 1.7–9.0) versus 0.5% (–0.4 to 1.4), respectively, with a difference of 4.8% (1.4–8.3; $p=0.0115$). The amount of necrosis was significantly lower in the ASC-enriched grafts than in the control grafts: 4.6% (95% CI 2.7–6.5) versus 16.1% (8.3–24.0), respectively, with a difference of 11.5% (3.4–19.6; $p=0.0105$). With respect to morphologically normal connective tissue, we also found a significantly lower amount in the ASC-enriched grafts than in the control grafts: 5.7% (95% CI 3.4–8.1) versus 16.4% (10.6–21.2), respectively, with a difference of 10.6% (3.9–17.4; $p=0.0060$; figure 5). There was no significant difference in vessel density between the ASC-enriched grafts and the control grafts: 20.86 vessels/mm² (95% CI 10.36–31.36) versus 26.32 vessels/mm² (13.55–39.08), respectively, with a difference of 5.45 vessels/mm² (–8.22 to 19.13; $p=0.39$; figure 5). No signs of malignant transformation were seen in any of the specimens.

Discussion

Our results indicate that ASC enrichment of fat grafts significantly improves graft survival. The high residual volume of more than 80% is encouraging with respect to the possible use of enriched fat grafts in reconstructive surgery. Compared with controls, the ASC-enriched grafts displayed higher amounts of adipose tissue and newly formed connective tissue and less necrotic tissue

after 121 days. The newly formed connective tissue was considered a product of the ASCs, as this tissue type is not usually observed in subcutaneous adipose tissue. These findings are in accordance with the fibroblast-like appearance and the differentiation capabilities of ASCs.¹⁸ To our knowledge, this is the first clinical study assessing the effect of lipofilling with fat grafts enriched with high-dose ex-vivo expanded ASCs (panel).

The residual volumes in the ASC-enriched grafts were high compared with controls and had little variance, despite being injected as a bolus. This finding is important when considering the potential use of ASC-enriched fat grafts for large-scale lipofilling, since the greater volume could broaden the indications for lipofilling to include recipient beds with otherwise too little well-vascularised tissue for transplant placement.¹⁷ Stromal vascular fraction-enriched fat grafts were injected in small quantities into multiple tissue areas (around 17.5 mL) in a recent study on facial contouring. The survival rate was only 64.8% (SD 10.2%) for the stromal vascular fraction-enriched grafts versus 46.4% (9.3%) for the control grafts.²² Therefore, the use of ASC-enriched fat grafts in a similar way is expected to lead to far more reliable and predictable results without the need for overcorrection.

We added ASCs at a concentration roughly 2000 times the physiological level.^{23,24} For comparison, in cell-assisted lipotransfer, the lipoaspirate needs to be four times the desired volume just to double the physiological ASC concentration in the fat graft because the ASC concentration in lipoaspirate is only half of the concentration in excised adipose tissue.¹⁶

In animal studies, the beneficial effect of ASCs on graft survival is thought to be because of improved early vascularisation, either as the result of ASC differentiation into endothelial cells^{13,14} or as the result of the immunomodulatory and trophic effects of ASCs.^{25,26}

Unlike the murine studies by Lu,¹³ Zhu,¹⁴ Chang,¹⁵ and colleagues, our study found no difference in vessel density between the ASC-enriched and non-enriched grafts, possibly indicating a state of vessel equilibrium at this timepoint. In vitro, adipocytes die easily under ischaemic conditions, whereas ASCs are activated and remain viable for up to 72 h.²⁷ Therefore, the coimplanted ASCs might have survived during the initial hypoxic conditions and gradually differentiated into fat cells to replace non-surviving mature fat cells. Future studies should seek to test this hypothesis.

There is an ongoing debate regarding the possible oncological risks, especially with respect to increasing vasculogenesis, after autologous fat grafting with or without the addition of ex vivo expanded ASCs.²⁸ With respect to vasculogenesis, after 4 months we found no increase in vessel density. Additionally, there are no data indicating that malignant transformation of expanded human mesenchymal stem cells (ASCs) occurs.^{18,29} Furthermore, more than 1000 patients in clinical trials

have received mesenchymal stem cells, and no serious adverse events have been reported.^{29,30} However, long-term follow-up of the recipients is needed.

We have described some technical problems interfering with the present study. These problems, however, do not affect the interpretation of the findings and are not relevant for the clinical use of the procedure.

The ASC-enriched fat grafts retained more than 80% of the initial bolus volume 4 months after the grafting procedure. This result shows that ASC graft enrichment can render lipofilling a reliable procedure, since the resorption rate, quality of tissue, and safety can be predicted. Therefore, the procedure could be an alternative to tissue augmentation, such as breast surgery with allogeneic material or major tissue flap surgery. Further studies should seek to apply the described method in relevant patient populations.

Contributors

S-FTK was the principal investigator. S-FTK and ABM drafted the report, and all authors contributed to review and revision. S-FTK, AF-N, JJE, RSO, M-LMT, CT, BSR, JK, MK, ED, and KTD designed the study. S-FTK, AF-N, and KTD wrote the grant applications. PVG generated the randomisation codes and designed the study database. S-FTK, AF-N, JJE, and KTD were responsible for recruitment. The surgical procedures were done by JJE, KTD, S-FTK, BSR, and PVG. All cell processing and laboratory procedures were done by S-FTK and PVG. Data collection was done by S-FTK, AF-N, M-LMT, and CT. ABM and S-FTK analysed the data, and produced all figures and tables. All authors have seen and approved the final version.

Conflicts of interest

We declare that we have no conflicts of interest.

Acknowledgments

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