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# Stem cell enrichment does not warrant a higher graft survival in lipofilling of the breast: A prospective comparative study☆,☆☆

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**KEYWORDS**
Fat transplantation;
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BEAULI;
Volumetry

**Summary** *Background:* Stem cell enrichment is generally believed to be of crucial importance for success in lipofilling for cosmetic breast augmentation. No comparative clinical studies have been reported to support this.
*Methods:* A total of 18 women underwent breast augmentation with water-assisted lipotransfer (WAL). In 10 of the cases, transferred lipoaspirate was enriched with stromal stem cells using the Celution® system (Cytori Therapeutics Inc., San Diego, Ca, USA). Magnetic resonance imaging (MRI)-based volumetric analysis was done preoperatively and 6 months after the procedure. To verify scientifically that stem cells were transplanted, samples of the transplanted tissues were processed in the laboratory to isolate the adipose stem cells (ASCs).

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*Results:* MRI volumetry revealed a volume survival of the whole (watery) graft of mean 54% (SD 7) in the WAL only and of 50% (SD 10) in the WAL with stem cell-enrichment patients. As centrifugation of the WAL grafts demonstrated an average adipose tissue of 68%, the average volume survival of adipose tissue itself was 79% (SD 13) in the WAL only and 74% (SD 14) in the WAL with stem cell-enrichment patients. This difference (4.5%) was not statistically significant (independent samples $t$ test, $p = 0.330$, 95% confidence interval of difference, 4.8, 13.9%).

*Conclusions:* Breast augmentation by lipofilling using WAL alone is faster, cheaper, has a lower risk of contamination and offers at least an equal take rate. We do not see any advantage in stem cell enrichment by the Celution® system in cosmetic fat transplantation to the breast.



After Illouz started in 1983 to infiltrate liposuctioned adipose tissue into the breast[1] and Bircoll and Novack published this approach for autologous fat transplantation in 1987,[2] it has later on been modified and refined in many ways. Coleman's lipostructure[3,4] is mainly based on centrifugation of the fat grafts before reinjection and was altered by Khouri to accelerate the process.[5] Furthermore, Khouri added external expansion. Other techniques such as the Shippert restrain from centrifugation in order not to harm the adipose tissue.[6] Like the LipiVage System®[7] with the Shippert method the separation of the water phase is facilitated by a filter. Another way to harvest fat grafts is by applying the water jet-assisted liposuction (WAL) method, where adipose tissue is mobilised by a pulsating water jet.[8]

*In vivo*, the number of adipose tissue-derived stem cells (ASCs) in adipose tissue is high.[9] Harvesting fat grafts by liposuction reduces the amount of ASCs.[10] This opens doors for supplementation of the lipoaspirate with stromal cells and stem cells of another aliquot of fat tissue. Supplementation aims to restore the amount of ASCs in the lipoaspirate to approach the amounts seen in native adipose tissue.[11] This method is called cell-assisted lipotransfer (CAL)[11] or stem cell-enriched tissue (SET) injections.[12]

While viability testing of fat grafts processed with various techniques has been performed *in vitro* in many studies,[7,13,14] information about volume survival in humans *in vivo* is scarce. Until now, only five publications that applied reliable methods for breast volumetry adequately report volume survival after autologous fat transplantation to the breast. Yoshimura evaluated the volume survival after CAL by three-dimensional (3D) surface analysis[11] and Khouri and Del Vecchio[5,15] applied magnetic resonance imaging (MRI) volumetry as described previously by Herold and Ueberreiter to analyse the outcome of autologous fat performed with the Berlin augmentation by lipotransfer (BEAULI) method, based on WAL.[16,17] It was demonstrated that MRI volumetry is exact and reproducible[16] and should be applied for qualitative and quantitative follow-up after autologous fat to the breast.[18] Three-dimensional imaging is very exact when analysing dummies, but when measurements are performed in human breasts, volume deviation still amounts to 60%.[19]

We performed a prospective study to find out if stem cell-enriched fat transplantation to augment breasts gives better results compared to traditional fat transplantation without stem cell enrichment. All patients were operated on with the WAL system and one surgeon. The results were analysed with MRI volumetry. To verify scientifically that stem cells were transplanted, samples of the transplanted tissues were further processed in the laboratory to isolate the ASCs.

## Patients and methods

This prospective, controlled research project was approved by the Ethics Committee of the University Hospital of Tampere (code R09171). The study was performed in a private clinic, Plastic Surgery Hospital KL in Helsinki, Finland, between September 2009 and November 2011.

### Patients and surgery

All healthy, non-smoking women with symmetric breasts seeking augmentation with fat transplantation were informed about the possibility of participating in the study. The patients who wanted to have stem cell enrichment had to pay extra for the cost of the consumables of the Celution® system, and they had to have some extra fat for enrichment. Both groups had free MRI before and after the procedure.

Breast augmentation with fat was performed by the first author in 18 patients between 2009 and 2011 (Table 1). In all patients the adipose tissue was harvested applying the WAL technique. In 10 patients (STEM group), additionally a stromal cell stem cell enrichment of the grafts was performed by using the Celution® 800/CRS system (Cytori Therapeutics Inc., San Diego, CA, USA). The control group (WAL group) consisted of eight patients without stem cell enrichment. Both groups included one patient with implant removal and consecutive fat grafting. All patients were non-smoking and healthy. In the stem cell group, the mean age was 51 years (29–58) and body mass index (BMI) was 23.4 kg $m^{-2}$ (20.3–32.5), and in the control group, the mean age was 39 years (33–63) and BMI was 23.4 kg $m^{-2}$ (20.3–25.9). All demographic data, grafted volumes and measured volumes are listed in Table 1. Basic patient characteristics are listed in Table 2. All patients had normal findings in a mammogram and ultrasound before joining the

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**Table 1** All demographic data of the patients of the study as well as grafted volumes, measured volumes and complications

| Patient | Age, Hormonal status | BMI | Total volume grafted right/left breast (ml) | Total volume fat grafted right/left breast (ml) | Volume for stem cell isolation (ml) | Earlier operations | Weight change (kg) | Removed implant size (ml) | Original breast volume right/left breast (ml) | Volume change right/left breast (ml) | Take of total grafted volume right/left breast (%) | Take of total grafted fat right/left breast (%) | Complications: nodules, lumps or cysts | Would I do it again? |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **STEM** | | | | | | | | | | | | | | |
| 1. | 46 M | 22.0 | 180/180 | 122/122 | 260 | WAL fat transfer | 2 | | 614/618 | 103/101 | 57.2/56.1 | 84.2/82.5 | 0 | no |
| 2. | 45 | 22.3 | 233/233 | 158/158 | 260 | | −2 | | 556/588 | 114/94 | 48.9/40.3 | 72.0/59.3 | 0 | yes |
| 3. | 46 | 23.5 | 210/215 | 143/146 | 253 | | 0 | | 634/657 | 84/83 | 40.0/38.6 | 58.8/56.8 | 0 | yes |
| 4. | 51 | 22.0 | 250/230 | 170/156 | 320 | | 0 | | 807/841 | 113/82 | 45.2/35.7 | 66.5/52.4 | 0 | yes |
| 5. | 53 M | 32.5 | 370/438 | 252/298 | 365 | | 0 | | 1298/1371 | 198/235 | 53.5/53.7 | 78.7/78.9 | CYST | yes |
| 6. | 51 P | 28.9 | 380/380 | 258/258 | 360 | | 6 | 140/140 | 1374/1347 | 232/237 | 61.1/62.4 | 89.8/91.7 | 0 | yes |
| 7. | 53 M H+ | 23.2 | 280/250 | 190/170 | 340 | | 2 | | 1010/1110 | 116/105 | 41.4/42.0 | 60.9/61.8 | 0 | unsure |
| 8. | 29 | 24.3 | 290/290 | 197/197 | 355 | IR 2 years earlier | 0 | | 718/754 | 123/143 | 42,41/49.3 | 62.4/72.5 | 0 | yes |
| 9. | 53 M H+ | 28.8 | 370/410 | 252/279 | 360 | Two FAR left breast | −1 | | 1309/1292 | 247/265 | 66.8/64.6 | 98.2/95.1 | 0 | yes |
| 10. | 58 P H+ | 20.3 | 230/270 | 156/184 | 240 | | 1 | | 640/559 | 141/132 | 61.3/48.9 | 90.2/72.0<br>**Mean 74.2**[a]<br>**Mean 72.4**[b] | CYSTS | yes |
| **WAL** | | | | | | | | | | | | | | |
| 11. | 38 | 25.7 | 260/300 | 177/204 | | WAL fat transfer | −4 | | 1057/1075 | 121/104 | 46.5/34.7 | 68.4/51.0 | 0 | yes |
| 12. | 58 P H+ | 22.4 | 330/300 | 224/204 | | Two FAR left breast | 0 | | 1017/1061 | 190/155 | 57.6/51.7 | 84.7/76.0 | 0 | yes |
| 13. | 63 P H+ | 20.3 | 300/300 | 204/204 | | | 1 | | 488/527 | 164/171 | 54.7/57.0 | 80.4/83.2 | 0 | yes |
| 14. | 39 | 22.2 | 300/300 | 204/204 | | | −2.5 | | 948/882 | 176/167 | 58.7/55.7 | 86.3/81.9 | 0 | unsure |
| 15. | 39 | 25.9 | 340/345 | 231/235 | | | 0 | | 775/839 | 146/136 | 42.9/39.4 | 63.2/58.0 | 0 | yes |
| 16. | 34 | 24.3 | 300/300 | 204/204 | | | 2 | | 665/673 | 189/193 | 63.0/64.3 | 92.7/94.6 | CYSTS | yes |
| 17. | 33 | 24.9 | 270/250 | 184/170 | | | 1 | | 862/872 | 175/154 | 64.8/61.6 | 95.3/90.6 | 0 | yes |
| 18. | 43 | 22.3 | 280/330 | 190/224 | | | 0 | 260/260 | 1300/1321 | 147/171 | 52.5/51.8 | 77.2/76.2<br>**Mean 78.8**[a]<br>**Mean 81.5**[b] | 0 | yes |

STEM; stem cell enriched lipotransfer, WAL; lipotransfer without stem cell enrichment, IR; implants removed, FAR; fibroadenomas resected, CYST; one <5 mm oil cyst, CYSTS; few <10 mm oil cysts, M; menopausal, P; postmenopausal, H+ hormone replacement therapy.

[a] Mean fat take (%) for all patients.

[b] Mean fat take (%) for patients with less than 5% weight change postoperatively (one patient excluded from both groups).

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**Table 2** Comparison of basic patient characteristics between groups.

| | WAL-STEM ($N = 10$) | WAL ($N = 8$) | $p$ value[a] |
|---|---|---|---|
| Age (years) | 51 (29–58) | 39 (33–63) | 0.183 |
| Menopausal | 6 (60%) | 2 (25%) | 0.157 |
| Preoperative BMI | 23.4 (20.3–32.5) | 23.4 (20.3–25.9) | 0.854 |
| Original breast volume right (ml) | 763 (556–1374) | 905 (488–1300) | 0.790 |
| Original breast volume left (ml) | 798 (559–1371) | 877 (527–1321) | 0.929 |
| Net fat grafted right breast (ml) | 180 (122–258) | 204 (177–231) | 0.349 |
| Net fat grafted left breast (ml) | 177 (122–298) | 204 (170–235) | 0.225 |

Values are median (range) or frequency (%), BMI; body mass index.
[a] Mann–Whitney *U* test or Fisher's exact test.

study. Informed consent and agreement were signed. Smokers, too thin patients or patients with visible asymmetry or any disease were excluded from the study.

## Liposuction – WAL

Liposuction for retrieval of fat was performed according to the WAL method, as described before, under local anaesthesia and light sedation.[17] The body-jet system was used in combination with the LipoCollector® (Human Med AG, Schwerin, Germany) (Figure 1). The tumescent solution containing 1 ml epinephrine 1:1000, 12.5 cc sodium bicarbonate 8 mval and 500 mg lidocaine each 1000 ml saline 0.9% was infiltrated 10–20 min before starting liposuction. A 3.8-mm steel cannula (yields particle size of maximum diameter 0.9 mm) was used; suction vacuum was set to 0.5 bar. Continuous rinse with tumescent solution at 37 °C was used throughout the liposuction procedure to assist mechanically and rinse the collected fat. Centrifugation was not used.

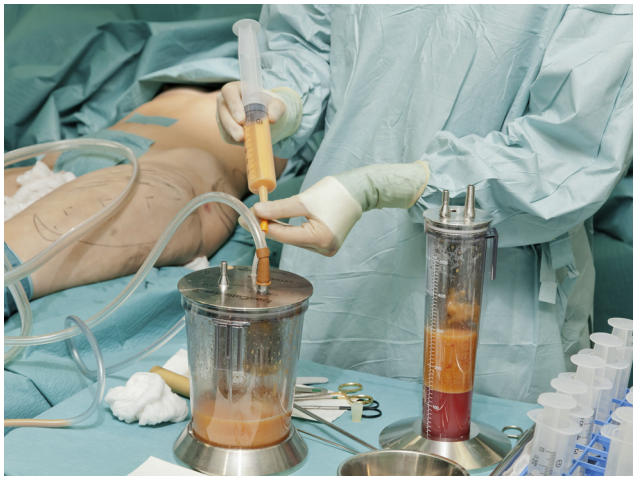

**Figure 1** The lipocollectors of the WAL (water-assisted liposuction) system (or Body-Jet®). The large one on the left can be used for collecting small to large volumes, and the narrow one on the right works best for small volumes. In both collectors, an inner filter collects remnants of connective tissue. The fat is automatically rinsed during harvesting, and only 10–15 min decantation in 50 ml syringe is required before transplantation.

## Celution® 800/CRS system and isolation of stromal fraction

Under sterile conditions the Celution® 800/CRS system® (Cytori Therapeutics Inc., San Diego, CA, USA) allows processing of the lipoaspirate gained from liposuction in the operating room; the risk of contamination and destruction of the cells due to transport to an outside laboratory is minimised (Figure 2). The automated process offers the possibility for standardised and reproducible isolation.

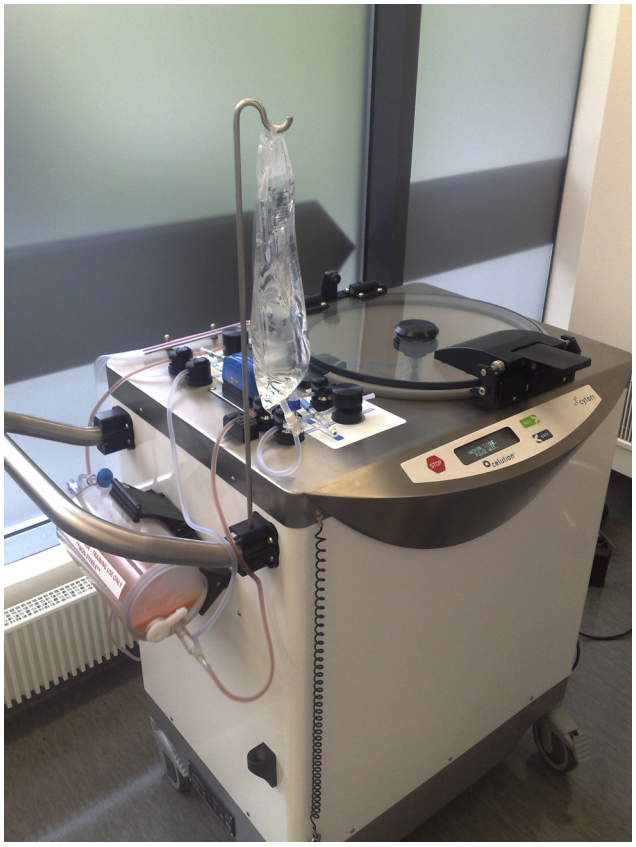

**Figure 2** The Celution system in action to isolate the stem cell rich stroma. The liposuctioned fat is first rinsed in a chamber on the left. The semiautomatic, isolated system is located in the operation theatre.

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Basically, this protocol follows the method described by Zuk et al.[20]

After harvesting with the WAL technique, the first part (240—360 ml in our series) is transferred directly into the Celution® System. Enzymatic digestion with several collagenases (Celase™ 835/CRS®) (Cytori Therapeutics Inc., San Diego, CA, USA) is started after washing and depletion of erythrocytes inside the chamber of the system. The stromal fraction, containing the ASCs, can be taken from the system after several cycles of automatic centrifugation and washing. Approximately 3—5 ml of ASC-rich stroma is gained and can be used to enrich another aliquot of lipoaspirate to produce a stem cell-enriched fat graft.[21] During the isolation process (over 2 h in our series), the ordinary fat graft was collected, decanted in 50-ml syringes and, after removing the visible fluid layer, mixed with the ACS-rich stroma. The mixture was transferred gently to 10-ml syringes (Figure 3) and injected immediately using a Celbrush™ injector® (Cytori Therapeutics Inc., San Diego, CA, USA) and a 2-mm blunt cannula. Via three 2-mm holes (inferolaterally, inferomedially and in the upper margin of areola) approximately one-third of the graft was injected in pectoral muscles and the retroglandular space and two-thirds subcutaneously, in very thin rows to yield as even a distribution of fat graft as possible.

**Figure 3** After decantation, fluid is removed from the syringe (left) and the fat is transferred to a 10 ml syringe, connected to the Cytori Celbrush® injector (right) for even distribution of the fat transplant.

## Analysis of MRI by radiologist

All patients received MRI scans before and 6 months after surgery to exclude complications. Examinations were performed using a 1.5 T clinical whole-body scanner (Philips Achieva R 3.2 Philips Medical Systems Nederland BV, The Netherlands). The patient was placed in a prone position. A breast case was used to prevent compression and deformation of breast tissue. Examinations covered the entire breast in the axial scan direction. No intravascular contrast agent was used. All MRIs have been analysed by a board-certified radiologist.

## MRI volumetry

All volume analyses were performed by a blinded independent examiner. Object marking was done using a WACOM Cintiq 12WX® liquid crystal display (LCD) (Wacom Company Limited Kazo-shi, Saitama-ken, Japan) graphic tablet and segmentation and volume analysis was done by the Brainlab® iPlan 3.1 neuronavigation software (Brainlab, Feldkirchen, Germany) (Figure 4). The selection of region of interest and process of MRI volumetry has been described previously.[16]

## ASC isolation and culture

To verify scientifically that stem cells were transplanted, samples of the WAL-assisted liposuction aspirate as well as the WAL-assisted stem cell-enriched liposuction aspirate using the Celution® system were further processed in the laboratory to isolate the ASCs by the method described previously.[20,22] Subsequently, the isolated ASCs were maintained and expanded in polystyrene flasks (Nunc, Roskilde, Denmark) in a medium containing Dulbecco's modified Eagle medium/Ham's nutrient mixture F-12 (DMEM/F-12 1:1; Invitrogen, Darmstadt, Germany) supplemented with 1% L-glutamine (GlutaMAX; Invitrogen, Darmstadt, Germany), 1% antibiotics (100 U $ml^{-1}$ penicillin, 0.1 mg $ml^{-1}$ streptomycin; Invitrogen, Darmstadt, Germany) and 10% human serum (HS; PAA Laboratories GmbH, Pasching, Austria) at 37 °C and 5% $CO_2$. When cells reached about 80% confluency, they were passaged and detached enzymatically using TrypLE SelectTM (Invitrogen, Darmstadt, Germany).

Cell sterility and endotoxins were tested at the Department of Public Health (University of Helsinki, Helsinki, Finland) according to methods described in the European Pharmacopoeia (Council of Europe, Strasbourg, France).

## Flow cytometric analysis of human adipose stem cell surface marker expression

To assess the stem cell immunophenotype of the isolated ASCs, the cells were harvested and characterised by flow cytometry (FACSAria™; BD Biosciences, Erembodegem, Belgium) as described previously.[22] Cell samples with 100,000 cells were single-stained with monoclonal antibodies against CD14, CD19, CD49d-PE, CD73-PE, CD90-APC and CD106-PECy5 (BD Biosciences, Erembodegem,

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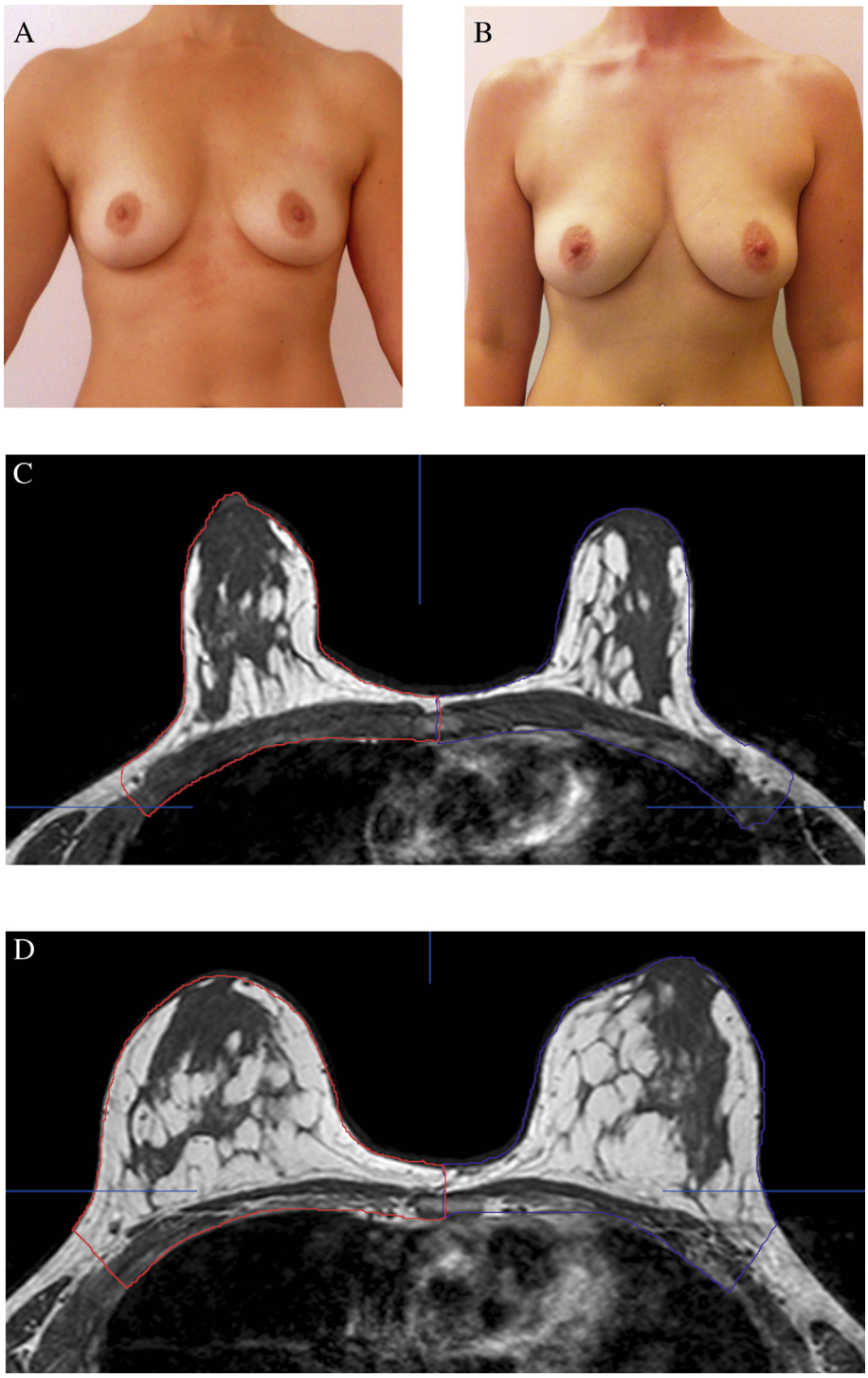


**Figure 4** The patient number 16 in Table 1, treated with WAL only without stem cell enrichment, before (A) and 1.5 years postoperatively (B), with MRI pictures before (C) and after 6 months (D). Notice the absolutely natural appearance of the breasts and increase in adipose tissue layer (white) surrounding the breast glandular tissue (black) and red and blue markings of the volumetric analysis.

Belgium); CD45-FITC (Miltenyi Biotech, Bergisch Gladbach, Germany); CD34-APC, HLA-ABC-PE and HLA-DR-PE (Immunotools GmbH, Friesoythe, Germany) and CD105-PE (R&D Systems Inc., Minneapolis, MN, USA). Analysis was performed on 10,000 cells per sample and unstained cell samples were used to compensate for the background autofluorescence. To facilitate the comparison, flow cytometric results obtained from ASCs isolated from adipose tissue fragments (en bloc) from six independent patients were added. The adipose tissue was harvested from

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patients undergoing elective surgical procedures in the Department of Plastic Surgery, Tampere University Hospital (Tampere, Finland).

### Statistics

The data were analysed with the help of PASW Statistics 18.0 for Macintosh (SPSS, Chicago, IL, USA). Data are expressed by mean (standard deviation or range) and median (range). Comparison between the groups was done with the Mann–Whitney *U* test (categorical or skewed data) or the independent samples *t* test (normally distributed data). When analysing fat takes, the data were pooled to form one group of cell-enriched breasts and another group of breasts without cell enrichment. Probabilities of $<0.05$ were considered significant.

The data from *in vitro* flow cytometry were analysed using one-way analysis of variance (ANOVA) with Bonferroni *post hoc* test used to compare the two liposuction products using GraphPad Prism for Windows, version 5.01 (GraphPad Software Inc., La Jolla, CA, USA). A *p*-value of $<0.05$ was considered statistically significant.

## Results

The operation time (2–3 h) was prolonged by 2–2.5 h in the stem cell-enrichment group, depending on the volume of fat used for enrichment. The mean amount of fat used for stem cell enrichment was 311 ml (range 240–365) (Table 1). The mean injected total volume/breast was 292 ml (range 180–438), depending mainly on the breast skin area (Table 1). There were no intra- or postoperative surgical complications. No complications or abnormal findings were detected in postoperative MRI, but in the long term, there was one case with few small oil cysts in both groups (patients #10 and #16 in Table 1). The cysts were clinically palpable and radiologically detectable (by ultrasound) approximately 1.5–2 years postoperatively. Both patients had originally small and thin breasts.

In all patients, the obtained volumetric change remained stable in the long term but was prone to changes in body weight. As the expectations and costs were higher in the stem cell group, some degree of disappointment with the final volumetric result was apparent in that group. After the study, all patients have been followed up annually clinically or by e-mail contact by the operating surgeon. Breast cancer has not occurred in either group.

The groups were statistically comparable (age, BMI, original breast size and transferred amount of fat, Table 2). MRI volumetry revealed a mean volume survival of the whole graft of 54% (SD 7) in the WAL only and of 50% (SD 10) in the WAL with stem cell-enrichment patients. As centrifugation of the WAL grafts demonstrated an average adipose content of 68%, the average volume survival of adipose tissue itself was 79% (SD 13) in the WAL only and 74% (SD 14) in the WAL with stem cell-enrichment patients. This difference (4.5%) was not statistically significant (independent samples *t* test, $p = 0.330$, 95% confidence interval of difference 4.8–13.9%). If patients with more than 5% change in body weight were excluded (one in each group), the average volume survival of adipose tissue was 81% (SD 11) in WAL only and 72% (SD 14) in WAL with stem cell enrichment. Again, although showing a strong trend, this difference (9.1%) was not statistically significant (independent samples *t* test, $p = 0.052$, 95% confidence interval of difference 0.1–18.3%).

As it became apparent that clinically, no benefit of stem cell enrichment could be obtained, the study was discontinued for ethical and economical reasons.

### Immunophenotype of ASCs

Flow cytometric analysis was used to compare the immunophenotype of ASCs isolated from WAL only and stem cell-enriched WAL with ASCs isolated from adipose tissue fragments (en bloc). Statistical analysis revealed no significant differences in the immunophenotypes of the different ASC populations and the results were in line with previously published results.[22–24]

## Discussion

During the last few years a lot of research has been carried out and hope has been put on stem cells on their use in clinical medicine. Stem cell-enriched fat is a magical concept and causes high expectations in us and in our patients. However, there are very few studies on the possible advantages of stem cells in breast surgery and specifically in breast augmentation. On the other hand, lipofilling of the breast has become a more or less routine procedure and new techniques have been introduced during the last years.

In this prospective study, we compared stem cell-enriched fat transplantation to breast with fat transplantation without stem cell enrichment. Here, we also used a rather new WAL technique in which tumescent liquid is infiltrated continuously with high pressure whereas fat is suctioned with low pressure and no centrifugation of the fat is used.[17] In principle, better results were expected with the additional amount of stem cells when compared to standard fat grafts.

Eto from Yoshimura's group presented data that aspiration of adipose tissue leads to a reduced adipose tissue stromal cell yield in comparison to excised adipose tissue, probably due to mechanical trauma.[10] To ameliorate the adipose tissue stromal cells in aspirated tissue and to compensate for the tissue damage of liposuction, CAL is an option to convert relatively progenitor cell-poor tissue into progenitor cell-rich tissue.[25] According to Suga et al., 100 ml of adipose tissue contains 100 million stem cells.[26] Thus, subcutaneous tissue in a healthy breast contains approximately 100–1000 million stem cells. Data presented by Cytori claim that 100 ml of adipose tissue processed with the Celution® system result in 25–40 million cells. Another study applying the Celution® system confirmed that an average of 295.176 cells can be isolated from 1 ml of adipose tissue, which means 30 million cells from 100 ml.[21] If enrichment increases the number of stem cells in the breast by 3–30%, is it of clinical significance in healthy tissue? Transplanted fat contains stem cells even without enrichment, depending on the trauma caused by the method of harvesting.[27]

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The angiogenic potential of ASCs is believed to be responsible for an improved vascularisation, providing a more hospitable bed for the transferred adipose cells. In regenerative indications such as ischaemic, radiation-injured tissue, Rigotti reported promising results with ASC-augmented fat,[28] where in most patients an amelioration of the wounds was observed. In our own experience, we were able to detect an improved tissue oxygenation by laser Doppler after treatment of patients with radiation ulcer with ASC-augmented fat grafts.[29] In a study consisting of 30 patients, ASCs have been used for regenerative therapy of facial hemiatrophy, pectus excavatus, gluteal soft-tissue defects and for breast reconstruction[12] as the angiogenetic potential of ASCs may also be useful in tissue augmentation.[30,31]

Moseley had stated in 2006 that although fat itself is inconsistent as a filler on its own, stem cells may enhance fat grafting as animal studies have shown a 2.5 times higher fat preservation rate by this approach in comparison to unprocessed plain fat grafts.[30] Yoshimura and co-workers found out in their experimental studies that CAL grafts survived 35% larger on average than non-CAL fat grafts.[32] Based on these findings, Yoshimura reported about CAL for cosmetic breast augmentation in 40 patients in 2008 with satisfactory clinical results.[11]

Every step in fat transplantation, harvesting, processing and transplantation — is important, but viability of the harvested fat cells is crucial. Gentle liposuction yields a transplant that is comparable to excised fat in viable stem cell count.[27,33] Any delay in transplanting the aspirate should be avoided. According to Sasaki, viability of 90% at 1 h after harvesting turns to 10% after 6—8 h.[8] The chances of survival are higher the less one manipulates the fat graft and the more quickly it is reinjected.[13] Whether there would have been a difference in the present study without the delay caused by the Celution® system remains unclear. When harvesting large amounts of fat, fast and gentle harvesting is not only a question of comfort but also of cell viability. As the WAL graft is rinsed and filtered automatically during liposuction, it can be transferred after short decantation, without processing and delay. The fluidity of the WAL fat graft enables easy injection with a 1—2 mm cannula without pressure and damage to fat cells. In addition, the consistent size of the WAL fat cell clusters (600—900 μm) helps the grafted fat microdroplets to survive as it is the optimum size for perfusion into the core of the cell clusters within the recipient area.[34] WAL might thus offer some benefits compared to the methods used in experimental studies, which might be a reason why no difference could be noted. To obtain even better uptake, we nowadays try to avoid local anaesthetics because of lidocaine toxicity.[35] Instead of general anaesthesia, most of our patients are operated in epidural anaesthesia.

Qualities of the recipient site may be limiting factors in WAL fat transplantation as in any method. Transplanted fat is vulnerable to pressure. Constricted skin with a very thin or scarred subcutaneous fat layer cannot sustain as large an amount of fat as healthy tissue, preferably with lax skin and thick subcutaneous fat layer. In the problematic cases, pre-treatment with. The Brava® system or other method of outer (or inner) expansion is probably more effective than trying to increase the amount of stem cells in the fat graft. Outer pre-expansion of the tight tissue envelope increases not only space and elasticity but also vascularity and, possibly, activates the existing millions of stem cells and thus enables transferring larger quantities of fat.[5] As Brava® is quite inconvenient to use, serial transfer is another option. As the WAL procedure is atraumatic, recovery is fast, which facilitates compliance with later procedures.[36]

Especially in breast surgery, the angiogenic potential of progenitor cell-rich tissue is associated with the risk of tumour induction. Although the *in vitro* findings of tumour induction[37] or of the interaction of ASCs with breast tumour cells[38] cannot be transferred into clinical setting directly, caution is inevitable and the authors believe that accurate follow-up is necessary. Benign subcutaneous small oil cysts may develop 1—2 years after lipotransfer and can cause anxiety but are easily treated by ultrasound-assisted aspiration, if needed.

Exact volume survival after CAL has not been analysed. Kim et al. present nice results in their actual study about fat cells differentiated from ASCs,[39] where progress control has been performed with 3D scanners and appealing long-term results were demonstrated.[39] Tiryaki analysed his 29 patients after SET injections, among them 15 with transplantation to the breast, by comparing pre- and post-operative images — an approach that is neither exact nor standardised.[12] Yoshimura was the first to present volumetric data after CAL to the breast.[25] Using 3D body scans, a graft take of 40—80% was demonstrated. Today, MRI is the gold standard for progress control after autologous fat transplantation as it is very exact and reproducible[16,40] and allows us to control volume survival as well as exclusion of complications or changes within the breast tissue as transplant recipient tissue as well.[18] Therefore, we applied MRI volumetry in a way it has been approved and described before.[16]

The WAL technique (BEAULI) has been demonstrated to be able to warrant survival rates of 72—76% of the transplanted fat.[16,17] This correlates with 52% of the whole, quite watery graft, as the WAL technique refrains from centrifugation in contrast to the Coleman or Khouri technique.[3,5] With these techniques, MRI volumetry has revealed graft survival rates of 64% and 82%, respectively. What kind of MRI volumetry protocol these authors used is not mentioned in their publications.[5,15] Nevertheless, we are very pleased that evaluation of different techniques of autologous fat transplantation reached a level that allows comparison of results based on the same methods for measurement.

The expected superiority of CAL, based particularly on the important *in vitro* studies and animal models by Yoshimura and co-workers, could not be accredited by our *in vivo* study in healthy humans, using the Celution® system. As it became apparent that using it is not beneficial, the study was discontinued for ethical and economical reasons, and as the number of treated patients remained too small for statistical significance the study remained unpowered. For future investigation, intra-individual comparison would be of high interest. In general, comparative, prospective clinical studies are still very rare, despite the vast amount of publications around stem cells. Publication of even negative results may help clinicians in today's

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enormous commercial assortment and pressure of economical interests. However, as known from the history of plastic surgery, aesthetic patients have always helped to pay for the development of innovations that later on have been adopted to treat serious diseases.

When our study was in the final stage, a joint task force of the two leading plastic surgery associations, the American Society for Aesthetic Plastic Surgery (ASAPS) and the American Society of Plastic Surgeons (ASPS), 9 May 2011, released a position statement that while there is tremendous potential for the future use of stem cells in aesthetic surgical procedures, the scientific evidence and other data are very limited in terms of assessing the safety or efficacy of stem cell therapies in aesthetic medicine. It is easy to agree with, as well as their recommendation that stem cell therapies in aesthetic and reconstructive surgery should be conducted within clinical studies under Institutional Review Board approval, including compliance with all guidelines for human medical studies. Stem cell enrichment still is an investigational procedure with hitherto unquantified risks.

## Conclusion

We found a high survival rate after WAL and cell enrichment in the presented patients, but not better than in patients purely treated with WAL, without stem cell enrichment. WAL alone is faster (90—150 min less), cheaper (cost of consumables for Celution® was over 3000 euros for each patient), theoretically safer (lower risk of contamination) and offers at least the same take rate. We do not see any advantage in stem cell enrichment by the Celution system® in cosmetic fat transplantation to the breast. The indications for CAL are rather to be seen in regenerative medicine.

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## Conflict of interest

None declared.

## Ethical approval

The research project was approved by the Ethics Committee of the University Hospital of Tampere, Finland (code R09171).

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