Detailed Experimental Procedures (SDC1)

Ethical and Regulatory Documentation

The Ethics Committee of the Balearic Isles authorized the research aspects of the present study. The clinical use of autologous adipose-derived cells was authorized by the regional Health authorities. The fat grafting preparation device (GID700) is both CE mark and USA 510(K) certified. The cell dissociation device (GID SVF-1) is a CE marked medical device available in Europe.

Patient Sample demographics (Table 1)

Patients were all female. Congenital and cosmetic indication Patients aged between 18 and 45 years. Post-oncological reconstructive patients involving cell-enhanced fat grafting ranged between 38 and 61 years of age. Age and BMI were found statistically indifferent between the low and high cell enhancement groups. All subjects retained a stable BMI throughout the follow-up period.

Surgical Planning and Patient selection

Care was taken to inform the patient on the limitations and risks of breast augmentation with cell-enhanced fat graft. Only patients younger than 45 years old and at low risk for developing breast cancer were selected.

Pre-operative data collection

Preoperative consent included authorization to perform the outlined surgery; obtain lipoaspirate and peripheral blood samples for research purposes; use of pictorial and unidentifiable data for publication and public presentations.

Surgical Indications and procedure-specific details (table 3)

Congenital volume deficits included 3 procedures of severe unilateral breast aplasia, and 3 cases of bilateral tuberous breast deformity in which cell-enhanced fat grafts were mainly implanted to restore the volume.

Breast volume deficits associated with cosmetic objectives represented the vast majority of the cases and included constitutional breast hypoplasia (n=33), volumetric replacement of compromised silicone implants following implant explantation (n=10) and silicone implant coverage (hybrid augment) (n=8).

All post-oncological reconstructive cases followed total mastectomy:
staged reconstructions (stage IIIA or lower) with skin envelope requirements were reconstructed with a TRAM flap (n=11) and immediate reconstruction patients (stage IIB or lower) underwent skin sparing mastectomies (n=9). No cases were performed following partial mastectomies.

Surgical Procedure

All patients underwent cell-enhanced fat grafting implanted within the subcutaneous, pre-pectoral or intramuscular[1] planes for a variety of congenital, cosmetic and reconstructive breast volume defects as listed in tables 1 and 2.

Briefly, the surgical procedure included all the following steps (figure 1), all performed within the same surgical time (3-5 hours).

Procurement of Lipoaspirated Adipose Tissue

All cases under general anesthesia started with infiltration of a modified Klein's tumescent solution Klein (modifications include Ringer's Lactate, no lidocaine; infiltrated volume equal to the target anticipated procurement volume). Patients underwent conventional power-assisted (Microaire®) liposuction using a cannula PAL-404LS connected to a conventional vacuum pump at an average pressure of 53.3 kPa (0.52 atm).

The initial harvest of raw lipoaspirate was consistently aspirated from the infra-umbilical area and flanks, and collected in the GID SVF-1 device for stromal vascular fraction isolation.

The disposable device SVF-1^{TM} depicted in FIGURE 1 consists of a plastic canister that holds a filter mesh that collects and strains the adipose tissue that immediately removes the fluid phase. The device was typically situated on the sterile field, interposed between the harvesting cannula and the waste canister.

Adipose tissue intended as the graft material was collected and processed in the GID700 device following manufacturer instructions.

Processed fat with low aqueous content was removed into 50 ml catheter tip syringes. This way all fat grafts were standardized for physical and physiological conditions before reinjection into the patient. Donor site areas treated were consistently limited to trochanteric, subgluteal areas, thighs, and knees.

Adipo-Derived Stromal Vascular Cell Isolation

The canister containing the adipose tissue was moved to the side table in the same operating room for processing. SVF isolation was carried out using the GID SVF-1 as described previously (8). Mean processing time was 60-70 minutes.

Cell Quality and Safety Assessment

All cell suspensions obtained were analyzed by image cytometry using a Nucleocounter NC-3000 (Chemometec, Denmark) for cell total count, viability, cell cycle and mitochondrial potential assays following manufacturer's instructions, and performed according to previously published data (8). No statistical differences in phenotypical composition were found when samples were segregated between those that generated low versus high cell enhancement fat grafts (data not shown).

Additionally (table 2), the cell suspension was assayed for endotoxin levels (within FDA and EMEA permitted intravenous range) and microbiological studies to address possible contaminations.

Addition (Enhancement) of Stromal-Vascular Cells to previously prepared fat grafts

The freshly isolated and resuspended adipose SVF cells were combined with washed fat graft (in catheter tip 50 mL syringes) using a catheter tip to Luer-Lok adapters (1-2 mL addition of resuspended SVF cells per every 50 ml processed fat graft, contents were mixed back and forth). Mean enrichment per mL of "dry" fat graft is tabulated in table 1.

Implantation

Cell-enhanced fat grafts were transferred to 20 mL luer-lock syringes. Two 2 mm stab skin incisions, located at medial and lateral ends of the infra-mammary fold, allowed introduction of injection cannulas and comfortable interweaved grafting passes at different planes. Long passes were executed laying no more than 2 mL of cell-enhanced fat graft per pass. Average grafted volumes are shown in Table1.

Reflow point:

Grafting was only interrupted in 7 occasions (3 implant coverage, 3 implant replacement and 1 aplasia), when tissue pressure exceeded the implantation pressure generated by normal manipulation of the fat graft syringes, thus provoking the fat graft "reflow" (extruding through access sites).

Intraoperative data collection

A 10 mL syringe loaded with 10 mL sample of the prepared cell-enhanced fat graft (figure 2, panel a) was spun at 400g during 5 min. The lipocrit (oil fraction) and aqueous phase percentage of the implanted cell-enhanced fat graft were recorded. Aqueous phase was quantitatively analyzed for triglyceride content, lactate dehydrogenase, hematocrit, and osmolarity using an automated analyzer (Dimension RxL Max Analyzer; Siemens, Munich, Germany). The data were expressed as units per mL of fat graft (SDC2)

A 500 µL aliquot from the obtained cell suspension was sent to the laboratory for cell quality and safety analyses

Breast Volume Measurement (3D imaging)

In order to evaluate clinical outcome, a three-dimensional (3D) imaging scan was utilized to quantify breast volume changes. The 3D digital breast surface images (or meshes) were obtained using an ARTEC MHT 3D Scanner and then superposed to measure the volume difference, using the scan's own software (figure 2).

In all cases, a 3-D scan was obtained in decubitus position pre and post-grafting. In 3 hypoplasia cases and 3 post-LD volume deficit cases, sequential scans were taken immediately after delivery of incremental aliquots of graft. 3-

D contour profile precision and accuracy was validated with MRI.

The 3-D volume change (increment) achieved by a given graft *immediately* after surgery was calculated and normalized to the total volume of graft implanted. We termed this ratio the "Intra-operative Volume Restoration Index" (IVRI) and was calculated with the following formula:

$$\text{IVRI} = \frac{\text{(Immediate Post-graft 3-D Breast Volume - Pre-graft 3-D Breast Volume)}}{\text{Volume of Graft.}}$$

To assess breast volume changes over time, we measured the 3-D volume of breasts at multiple post-operative time points (from 7 days to 18 months). These measurements were then normalized to the breast volumes measured *immediately* after grafting. We have termed this ratio of long-term breast 3-D volume to immediate post-graft volume as the POVRI (post-operative volume retention index). The POVRI indicates the breast volume changes overtime.

Graphs and Statistical Analysis

All graphs, plots and statistical analysis have been carried out using GraphPad Prism 5.0 Software (GraphPad Corporation, San Diego). Error bars represent SD and not SEM.