Efficacy and Safety of Cell-Enriched Fat Grafting in the Breast

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6.1 Introduction

Fat transfer is a very useful technique in both cosmetic and reconstructive plastic surgery. A recent survey showed that approximately 80 percent of plastic surgeons have used fat grafting in their practice [1].

Since its first introduction in the late 1990s, its popularity has spread rapidly and the original technique has undergone several modifications to improve its efficacy.

Fat grafting has been used for facial contouring, post-traumatic deformities, radiation damage, congenital anomalies, and burn injuries.

Breast surgery is probably the best application for fat grafting (Table 6.1). It can be used for correction of imperfections in breast reconstruction with implants or in hybrid breast reconstruction, treatment of irradiated breast, correction of scar retraction after quartectomy, and breast augmentation for cosmetic purposes or capsular contraction [2–5].

One of the most important problems linked to fat grafting is its resorption rate that can be variable and unpredictable. References note that the resorption rate can range from 20 to 80 percent of the grafted tissue which necessitates multiple repeat procedures [6–9].

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Today, we know that the regenerative function of fat transfer is mainly mediated by adipose-derived stem cells (ASCs). ASCs have been identified as a well-defined cellular line with the ability to differentiate in mature mesenchymal cells. This has led to fat grafting becoming not only a filler but also a regenerative tissue for a lot of indications where it is able to improve both the volume and the skin quality, tissue trophism, and vascularity [10].

The interaction between the fat graft and the recipient site has been the object of several studies. In particular, most of these have identified three zones from the periphery to the center of the graft [11].

- *The surviving area:* adipocytes survive thanks to the best oxygen intake.
- *The regenerating area:* adipocytes die while adipose-derived stromal cells survive, and dead adipocytes are replaced with new ones thanks to the ability of ASCs to survive in lower oxygen conditions compared to mature adipocytes.
- *The necrotic area:* both adipocytes and adipose-derived stromal cells die.

It is clear that fat graft, thanks to this variety in terms of cellular composition, can act as a very complex tissue compared to other grafts, with different actions depending on the recipient site conditions. In addition, not only is the

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Table 6.1 Fat grafting indications in breast surgery

Breast reconstruction	Aesthetic breast surgery
• Breast Implant Reconstruction – *Filling contour irregolarities* – *Improvement of implant coverage* • Breast Conservation Therapy – *Correction as asymmetries, scar retraction and other contour deficits* • Total Breast Reconstruction – *Brava system* – *Reverse expansion* • Treatment of Irradiated breast • Miscellaneous – *Correction of asymmetries, scar retractions and* – *Other contour deficits in both autologous and implant breast reconstruction*	• Primary Breast Augmentation • Primary Implant Breast Augmentation – *Filling contour irregolarities* – *Improvement of implant coverage*
	Congenital anomalies – Poland Syndrome – Pectus Excavatus – Thoracic Hypoplasia

composition of fat graft in terms of regenerative cells important for obtaining the best grafting, but also the surgical technique must obtain the maximal contact surface between the graft and the recipient site (lipostructure) to ensure the best oxygen intake throughout the graft. Fat harvesting technique has a crucial role for a successful grafting considering that liposuction has a traumatic effect on adipocytes that can affect their vitality. Each step of this process must be carried out effectively in order to maximize cellular vitality.

The first large contribution to this methodology was from Coleman who proposed in 1997 his technique, the current standard, that low-pressure lipoaspiration, low-speed centrifugation, and other standardized steps allow for decreased cellular damage for adipocytes which increased graft vitality [12].

The fat resorption rate, however, continued to be inconsistent and unpredictable which led to researchers looking for methods to increase the number of ASCs in the fat graft.

Initially, some surgeons experimented with different types of growth factors such as insulin, VEGF, or PRP in combination with fat but none of these techniques showed a clear utility [13–16].

In 2006, Matsumoto et al. described for the first time the CAL (Cellular-Assisted Lipotransfer). In particular, they enriched the fat with SVF (Stromal Vascular Fraction) extracted from the lipoaspirate (which contains ASCs, endothelial cells, and other mesenchymal cells such as pericytes) in order to improve the survival rate of the grafted tissue [17].

Since then, several studies have demonstrated CAL efficacy [18–24]. The clinical application, however, is still not widespread due to few randomized clinical trials and the low level of evidence and safety data in the literature. In this chapter, we analyze the CAL in terms of techniques in breast surgery, its efficacy, and safety in a clinical application.

6.2 Cell-Assisted Lipotransfer

CAL (Cell-Assisted Lipotransfer) is a procedure through which the lipoaspirate is enriched with ASC or SVF in order to improve the regenerative function of the fat.

The resorption rate of the fat has always been a challenging problem for many reasons, especially the need to repeat the procedure several times.

Microscopical analysis of the fat, as demonstrated by some studies, showed that the suctioned fat has approximately half of the ASCs compared to the excised fat [25]. This can be explained by the fact that ASCs generally are more represented close to vascular structure and are, for this reason, generally preserved by the cannula. Moreover, a substantial number of ASCs go into the liquid part of the lipoaspirate.

The CAL stems from the need to address this problem using a part of the lipoaspiration as a reservoir of ASCs to enrich the material which acts like a living scaffold for ASCs (Fig. 6.1).

In particular, selective ASC extraction can only be performed through enzymatic digestion of the lipoaspirate and subsequent expansion in culture in a GMP (Good Manufacturing Practice) laboratory. This time-consuming process makes it impractical for clinical use and necessitates two separate surgical procedures (one for fat harvesting and one for fat grafting).

Intraoperative techniques (most commonly non-enzymatic) allow to extract the whole SVF which includes ASCs, MSC, HSC, Treg Cells, Pericytes, mast-cells, complex microvascular beds (fibroblasts, WBC, dendritic cells, intra-adventitial smooth muscular-like cells, etc.) and are more suitable for clinical applications due to their simplicity and rapidity of execution.

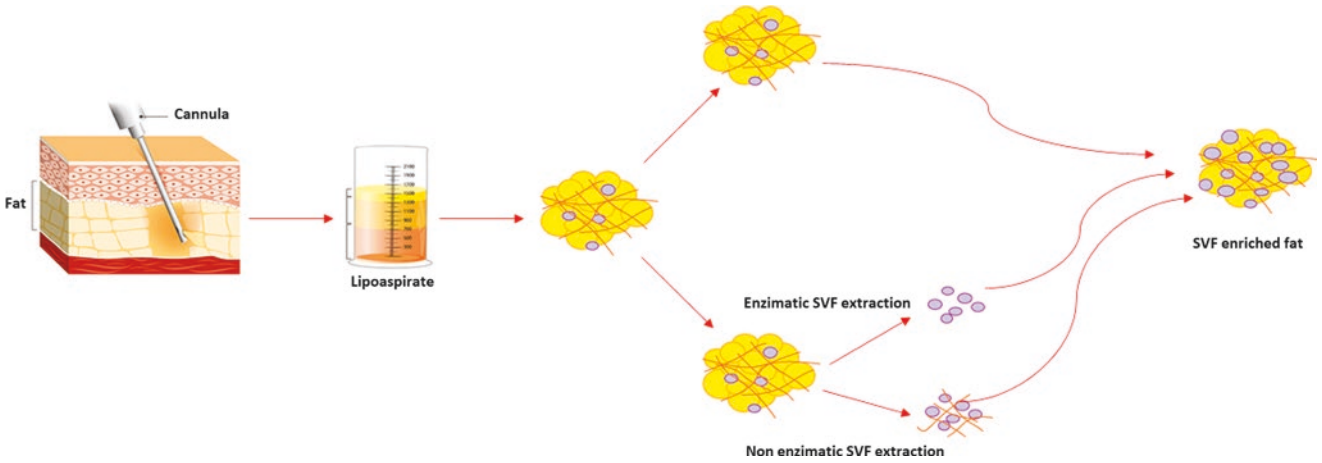

Fig. 6.1 Cell-assisted lipotransfer procedure

6.3 Stromal Vascular Fraction (SVF)

Zuk et al. in 2001 and 2002 were the first to identify the complexity of the cellular population found in adipose tissues [26].

Since the mid-late 2000s, many researchers and clinicians began to understand the complexity, contents, and paracrine functions of the adipose-derived stromal vascular fraction. We now know that it is composed of a highly heterogeneous population of cells: MSC, HSC, Treg Cells, Pericytes, mast-cells, complex microvascular beds (fibroblasts, WBC, dendritic cells, intra-adventitial smooth muscular-like cells, etc.). These cells are linked to each other to form a complex extracellular matrix [27].

If ASCs can be defined as the most important element for the regenerative function of fat graft, their vitality and their regenerative function are closely linked to this complex system of interaction. Fat harvested via en bloc excision delivers mature adipocytes and their attached progenitor cells (pre-adipocytes of presumed unipotent abilities as near terminally differentiated cells) which represent another group of secretory-capable cells within the grafted microenvironment. Appreciation of the importance of the complex, three-dimensional matrix found in adipose tissues has led many to consider the intact, non-manipulated transplantation of such tissues and their accompanying cellular elements may be advantageous.

6.4 Adipose-Derived Stem Cells (ASCs)

MSCs (mesenchymal stromal cells) were described for the first time by Friedenstein in 1970 in the bone marrow [28]. Decades later, we know there are several types of MSCs in many organs and tissues, including adipose tissue.

ASCs, first characterized in 2001, show a higher yield compared to bone marrow stem cells and their extraction is very easy thanks to the low invasiveness of liposuction compared to bone marrow extraction. They can differentiate in several cellular types of the mesenchymal line as adipocytes, osteoblasts, and chondrocytes; express cellular markers similar to other mesenchymal cells like CD105, CD73, and CD90; and are negative for CD31 and CD45. They have regenerative function in many aspects thanks to their angiogenetic, antifibrotic, and immunomodulatory effect. In addition, thanks to the release of paracrine modulators, they can improve the resistance to hypoxic conditions [10].

All these factors put ASCs at the center of scientific interest in regenerative surgery in the last 20 years and research in this field is still very active.

6.5 Cell-Enriched Fat Grafting in the Breast

6.5.1 Surgical Technique

Breast fat grafting procedure starts from a precise analysis of the defect we want to correct or of the breast volume we want to reach depending on the indication.

The patient is marked in the standing position where areas of contour irregularity can be most clearly seen due to shadows created by overhead lighting.

The surgeon estimates the amount of fat needed for grafting and selects one or more donor sites. Donor site selection includes any area that has sufficient fat to donate, typically the abdomen, hips, and thighs. The site selected can be based on ease of harvest and patient preference, since no studies have demonstrated superiority of different sites in graft survival [29].

Standard liposuction infiltration is performed with a dilute epinephrine and anesthetic solution to minimize blood loss (Klein solution).

6.5.1.1 Lipoaspiration

There are several different techniques to perform lipoaspiration. The author's preference is the Coleman technique. The main difference in this step when performing CAL compared to a standard lipofilling is in the amount of lipoaspiration. The volume must be approximately double the amount of final enriched fat required since half of the fat will be used to extract the SVF for enrichment.

The Coleman technique involves the use of a cannula which has a blunt tip and two adjacent holes. This is connected to a 10-mL syringe in order to apply a lowest suction compared to a larger syringe. As the cannula is pushed through the harvest site, a combination of the curetting action of the cannula and the negative pressure pulls fat into the syringe [30].

At this point, standard Coleman technique would proceed with centrifugation of all the lipoaspirate at 3000 rpm for 3 min. When performing CAL, however, the lipoaspirate is divided in two equal parts: one for SVF/ASC extraction and the other to be enriched, typically after centrifugation.

After centrifugation, the lipoaspirate components are separated by density. The upper level of oil (ruptured adipocytes from aspiration) is removed by pouring it off and then removing any remainder with absorbent wicks of Telfa (Kendall). The bottom, the densest level, contains blood and infiltration solution and is removed by briefly removing the cap and allowing it to drain. After this, the fat, ready to be enriched, is placed in ice [30].

6.5.1.2 SVF and ASC Extraction

There are several techniques for extraction of ASCs or SVF [31].

ASCs can be extracted from lipoaspirate and expanded in culture by submitting the fat to several steps of enzymatic digestion, washing, and manipulations in a GMP laboratory. It is a time-consuming process that requires a two-step surgery which makes it impractical for clinical use but is considered, despite this, the gold standard technique for ASC extraction.

There are several kinds of intraoperative extraction techniques that make the process easier and faster and can be divided into enzymatic and non-enzymatic techniques [32].

Intraoperative techniques allow to extract the whole SVF, not only ASCs. In particular, they allow to obtain a cellular SVF (cSVF) or a tissue SVF (tSVF) depending on the technique. Enzymatic techniques acting as digestion of the extracellular matrix generally allow one to obtain a cellular SVF. Non-enzymatic techniques act as a mechanical separation of the lipoaspirate components and generally do not affect the extracellular matrix, giving a tissue SVF that has a greater volume compared to cellular SVF [32]. In addition, mechanical techniques are faster than enzymatic techniques.

6.5.1.3 Intraoperative Enzymatic Extraction

Intraoperative enzymatic extraction can be performed in several ways. Typically, after lipoaspiration the fat for SVF extraction is washed several times with PBS solution (phosphate-buffered saline) and is put under 0.075% collagenase solution at 37.8 °C in a shaker bath for 30 min. It is then washed several times with PBS solution [33].

The collagenase action is inactivated with an equal volume of 10% fetal bovine serum (FBS) and the infranatant is centrifuged for 5 min at 1200 × *g*. The cellular pellet is then resuspended in 10% FBS and passed through a 100-μm mesh filter to remove debris.

At this point, the SVF extracted and the fat are gently mixed and after 10–15 min the SVF-enriched lipoaspirate is placed in the injection syringe.

6.5.1.4 Intraoperative Non-enzymatic Extraction

There are several protocols for non-enzymatic extraction of SVF from lipoaspirate (FAT, FAST, FEF, LIPOGEMS, etc.). Most of these procedures require a mechanical manipulation of the fat with different steps of filtration or centrifugation depending on the type of technique.

For example, Van Dongen et al. proposed in 2016 an SVF extraction performed through 3000 rpm centrifugation, passing through a 1.4-mm opening and then a second centrifugation at 3000 rpm [32].

Domenis et al. performed a filtration through a filter bag with a 120-μm filter and then a 400-*g* centrifugation [34].

The nanofat technique described by Tonnard et al. has been used as a SVF extraction technique (passing adipose tissue through a female-to-female Luer-Lok 30 times and filtering) [35].

As mentioned above, mechanical extraction of SVF leads to a tSVF that has a greater volume compared to cSVF. After the extraction, the SVF is used to enrich the lipoaspirate as mentioned previously.

6.5.1.5 Fat Injection Technique

Different cannulas are used for injection than for harvesting. Typically, the injection cannula is a blunt cannula with a single hole for precise fat deposition and is available in different lengths and flexibilities.

Droplets of fat grafts are deposited with each pass as the cannula is withdrawn and in multiple tissue planes after multiple passes. Regular distribution of the fat is essential to successful fat grafting since a wider surface of contact between the graft and the donor allows a better vascular supply. The total injected amount is calculated to not over graft the areas to treat, since it does not improve grafting and increases the formation of oil cysts.

The fat grafting technique can be different depending on the surgical indication.

Depressions are generally treated with multiple tunnels in a crosshatch fashion, while ridges and rippling respond best to a few long tunnels below the longitudinal depression. Depressed areas or those with scar retraction may benefit from scar release with specialized cannulas that have cutting edges.

For cosmetic breast augmentation, fat can be placed in different planes in order to enhance the volume, generally in the fatty layers on, around, and under the mammary gland. Some authors also infiltrate into the gland and in the pectoralis muscle but is this author's opinion that these procedures can lead to significant complications such as hematoma or fat embolism when performed in the pectoralis muscle or damage to gland ducts after intraglandular injection with risk of mastitis, infection, and excessive post-operative swelling. In fact, we generally use fat grafting alone only for mild cosmetic breast augmentation while very often in combination with breast implants to improve the cosmetic result of breast augmentation and to enhance implant coverage.

In post-oncological patients, fat grafting can be used as mentioned above to improve implant coverage as well as prevention and treatment of implant rippling (Figs. 6.2 and 6.3).

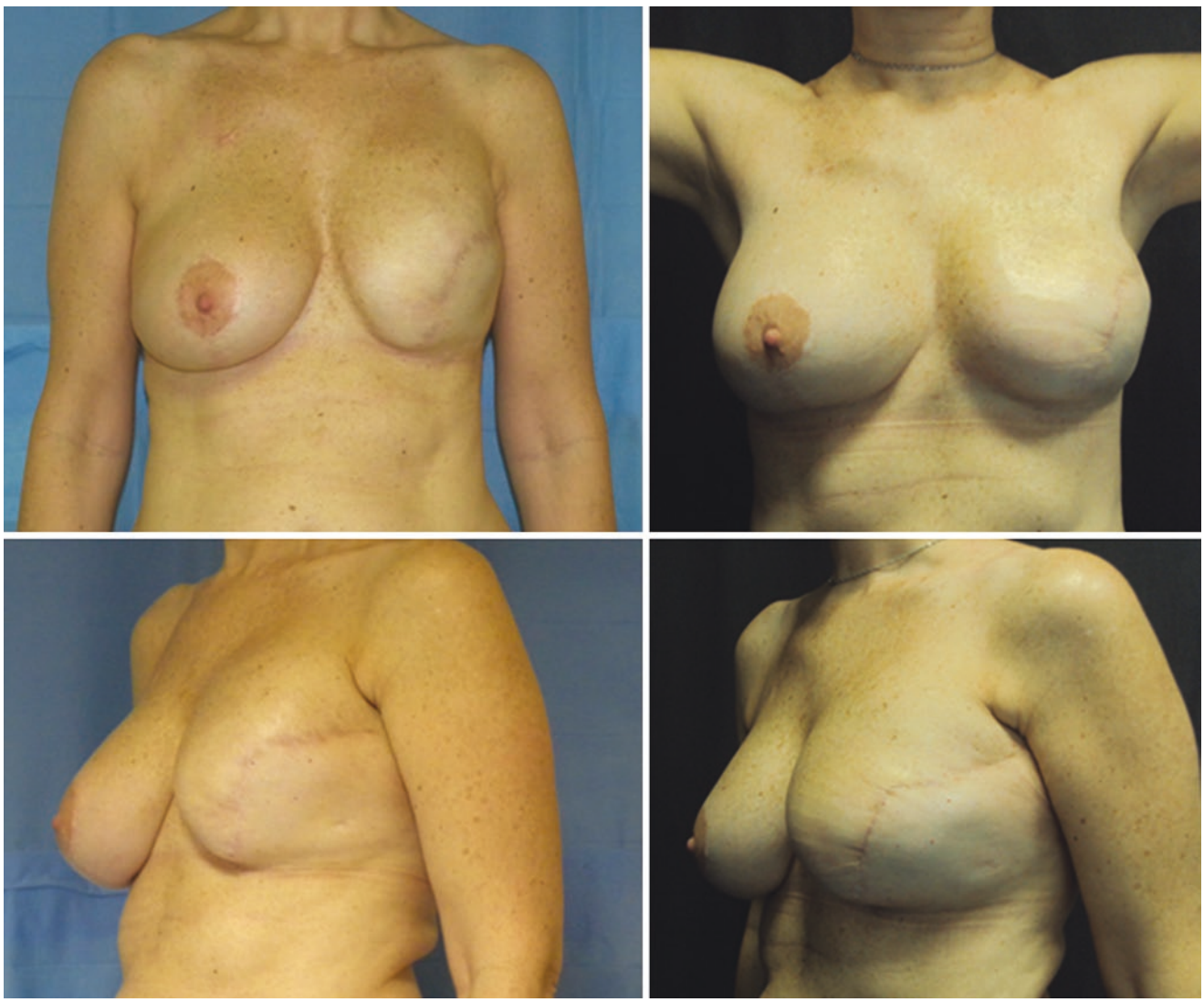

Fig. 6.2 Clinical case: fat grafting on breast implant reconstruction. One year post-op

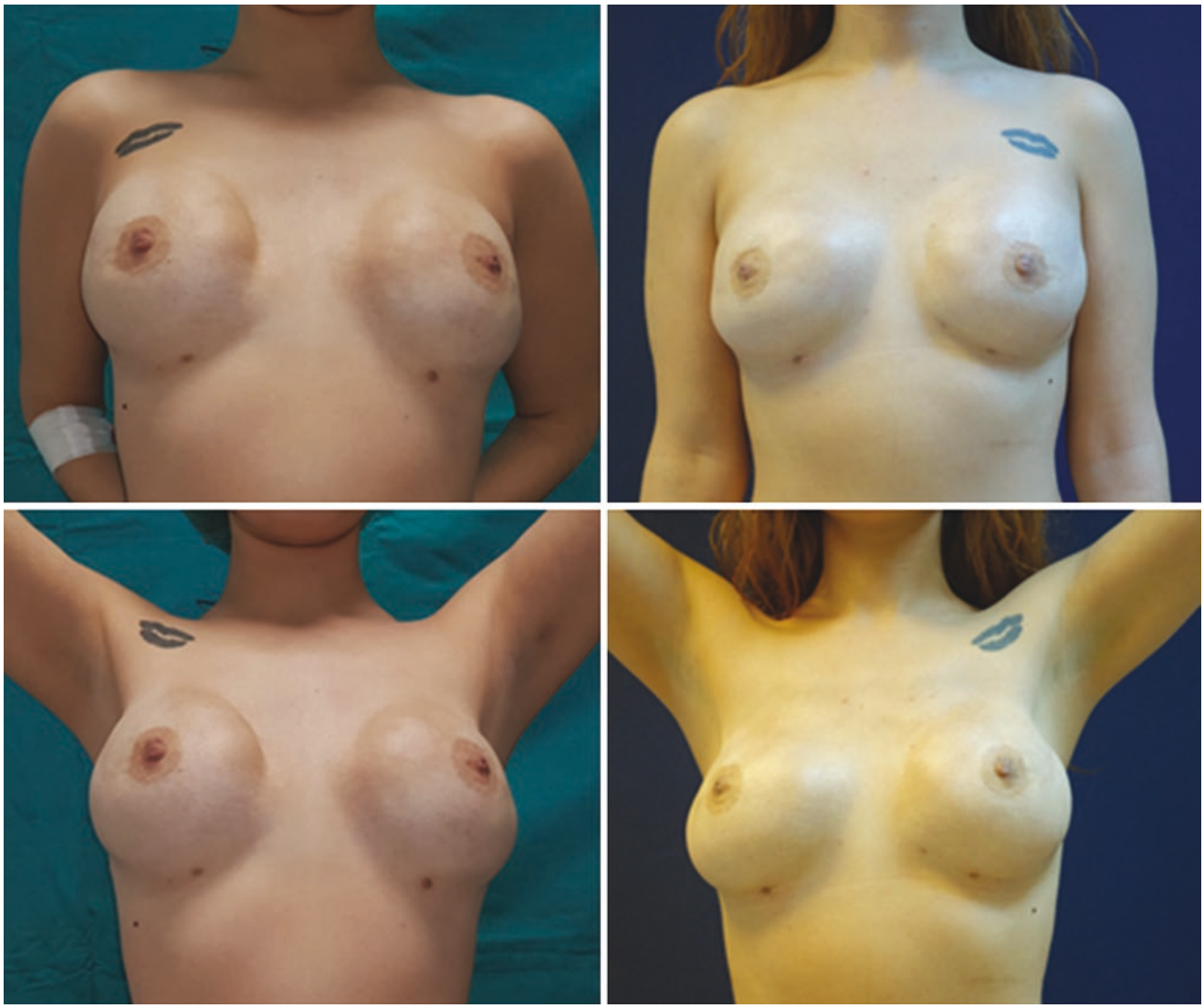

Fig. 6.3 Clinical case: bilateral breast implant reconstruction after preventive mastectomy. One year post-op after breast implant revision and fat grafting

Another common indication is to treat specifical deformity consequently to breast-conserving surgery treatments (BCS) as quartectomy or nodulectomy. These cases illustrate the dual benefits of fat graft on both the volume defect and on the scar retractions that are very common after BCS thanks to its antifibrotic effect (Figs. 6.4 and 6.5).

Finally, fat grafting can be a useful technique in all clinical conditions that require the correction of small asymmetries of the breast in both cosmetic and reconstructive context (Figs. 6.6 and 6.7).

6.5.2 Efficacy

Several studies demonstrated that CAL is a useful technique which can significantly reduce the fat resorption rate.

Two studies selectively investigated the efficacy of fat enriched with cultivated ASCs comparing it to traditional fat grafting. Koh et al. observed a significant reduction of fat resorption rate in the CAL group compared to the control group (20.59% versus 46.81%) [36].

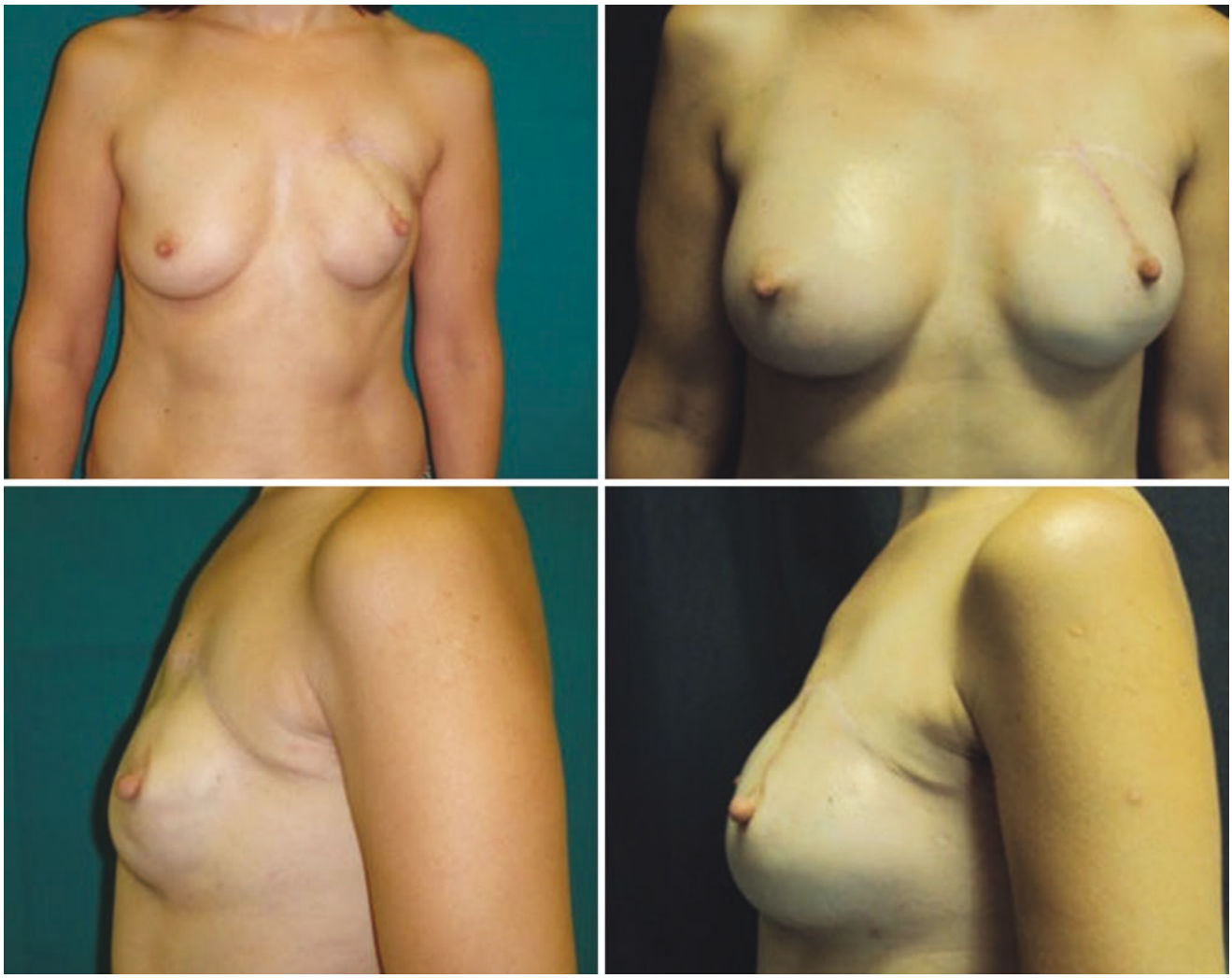

Fig. 6.4 Clinal case: fat grafting for scar retraction and asymmetry correction after BCS. Six months post-op

Similarly, Kolle et al. showed a fat survival rate of 80.9% for ASC-enriched graft compared to the 16.3% of traditional fat grafting, yielding a relative survival improvement of 5.0 [19].

Several studies investigated the general efficacy of CAL (in terms of both ASCs and SVF enrichment) compared to traditional fat grafting.

Zhou et al. recently performed a systematic review with meta-analysis on 17 previous studies, a global case series of 387 cases, and showed an improvement from 45% to 60% of fat survival rate with CAL compared to traditional fat grafting.

Furthermore, this improvement appeared to be even more significant for fat grafting in the face area and able to reduce the number of surgical operations, compared to breast fat grafting [18].

Laloze et al. performed a similar review expanding the case series but obtained slightly different results. They showed a fat survival rate of 64% for CAL vs 44% of traditional fat grafting with no differences in terms of efficacy for different injection sites. However, this improvement appeared to be significant only for small injection volumes (<100 mL) and, in addition, CAL showed a higher rate of complications compared to traditional fat grafting and did not reduce the number of surgical procedures [37].

Li et al. performed a similar review with meta-analysis comparing the efficacy of CAL with traditional fat grafting in the breast and showed a fat survival rate significantly higher in the CAL group. However, analyses of the subgroups comparing the SVF-enhanced fat graft with the conventional fat grafting noted no significant differences in fat survival rate, suggesting that the fat survival rate improvement observed might regard only ASC enrichment [38].

This apparent uncertainty can be partially explained by the lack of a standardized procedure that is universally

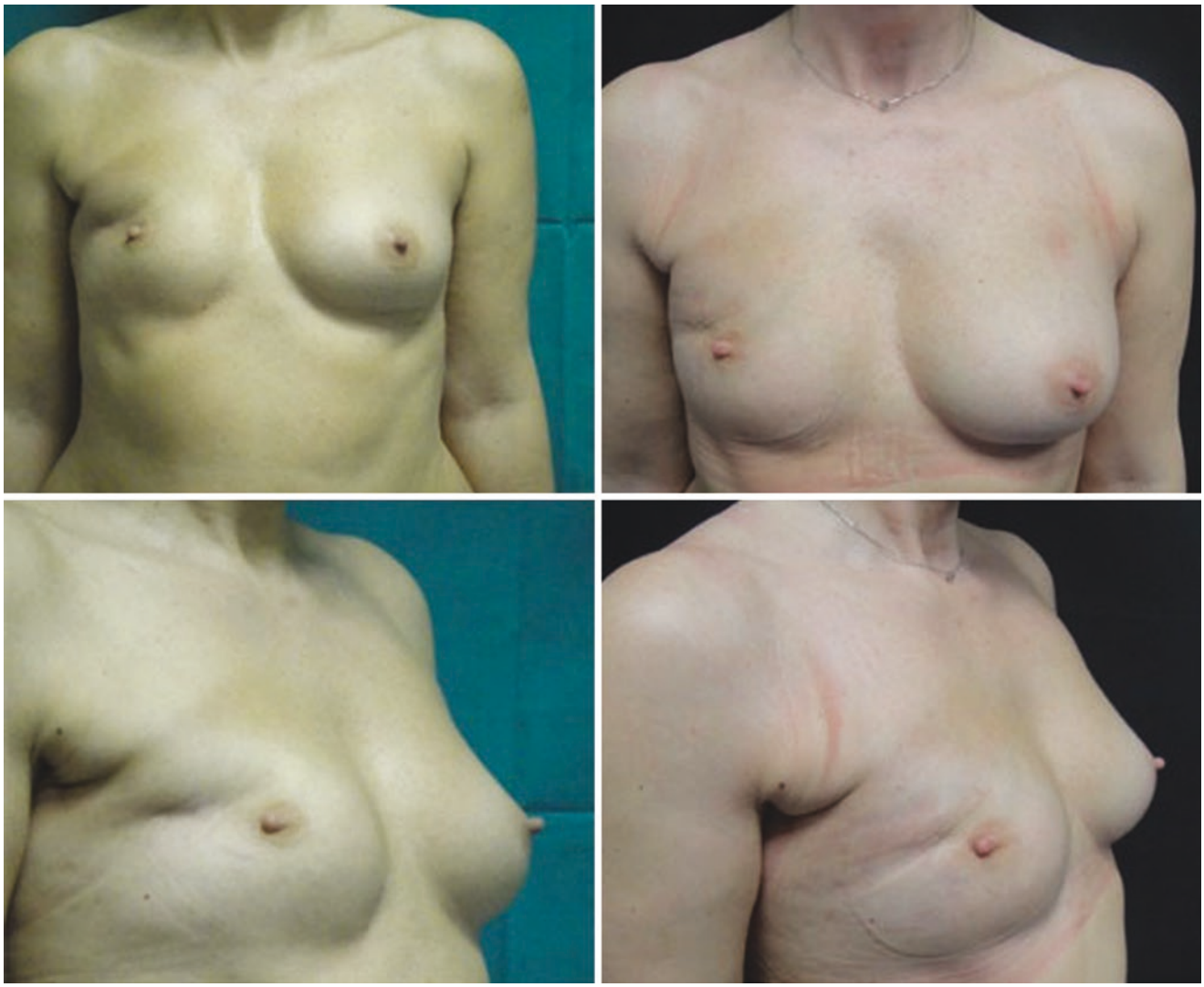

Fig. 6.5 Clinical Case: fat grafting for scar retraction and asymmetry correction after BCS. Eight months post-op

accepted by the scientific community. At the moment, several different processing techniques are described, each one with different outcomes [20, 22, 36].

In addition, some of these trials evaluated the fat survival rate through imaging techniques as MRI or Ecography, while other trials through patient satisfaction or clinical evaluation, creating even more the variability of the results [23].

By now, there is no evidence that any intraoperative isolation procedure could be designated as preferred procedure for isolating SVF [32].

Enzymatic and non-enzymatic procedures had comparable results as it comes to cell yield, viability, and SVF composition. Non-enzymatic isolation procedures produced end products that had greater volumes (tSVF) than the pellets (cSVF) of the enzymatic isolation procedures. The results of intraoperative isolation procedures are comparable with those of the gold standard, the collagenase-based non-intraoperative isolation protocol. Since intraoperative isolation procedures are less time-consuming, but as efficient as the non-intraoperative isolation protocol, the use of intraoperative isolation procedures seems to be more suitable for clinical purposes [32].

Finally, most of the countries have legislative problems with the manipulation of stem cells for a collagenase-based non-intraoperative isolation protocol, with the necessity of a GMP (Good Manufacturing Practice) laboratory, which is generally not present in most clinical centers.

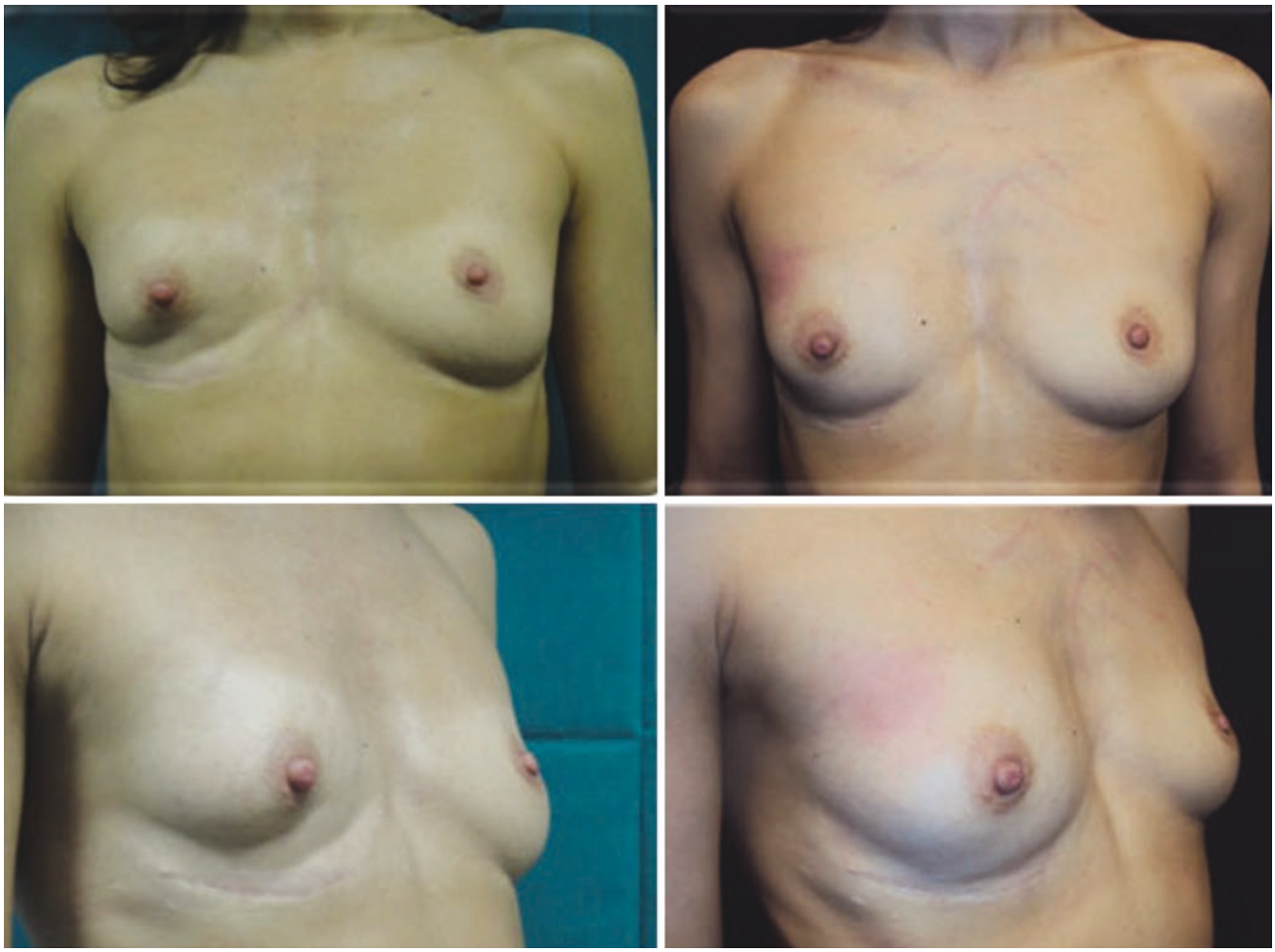

Fig. 6.6 Clinical Case: fat grafting for correction of breast contour irregularity. One year post-op after two procedures

6.5.3 Complications

Some studies showed a higher complication incidence with CAL compared with traditional lipotransfer (8.4% vs 1.5%), while others showed that this increased complication rate appeared to be significant only for breast fat grafting [18, 37].

Currently, the majority of the studies comparing CAL with traditional fat grafting did not show any significant difference in terms of classical complications like oil cysts, infections, fat necrosis, calcifications, nodules, and fibrosis.

6.5.4 Oncological Safety

Today, it is generally accepted by the scientific community that fat grafting is a safe technique when cautiously performed and, most importantly, when performed after complete cancer remission [39].

The subject of CAL is obviously still much debated due to the enhancement of its regenerative effect and the greater rate of ASCs.

Several in vitro trials demonstrate that ASCs can promote tumor growth and increase the risk of cancer recurrence [40–42].

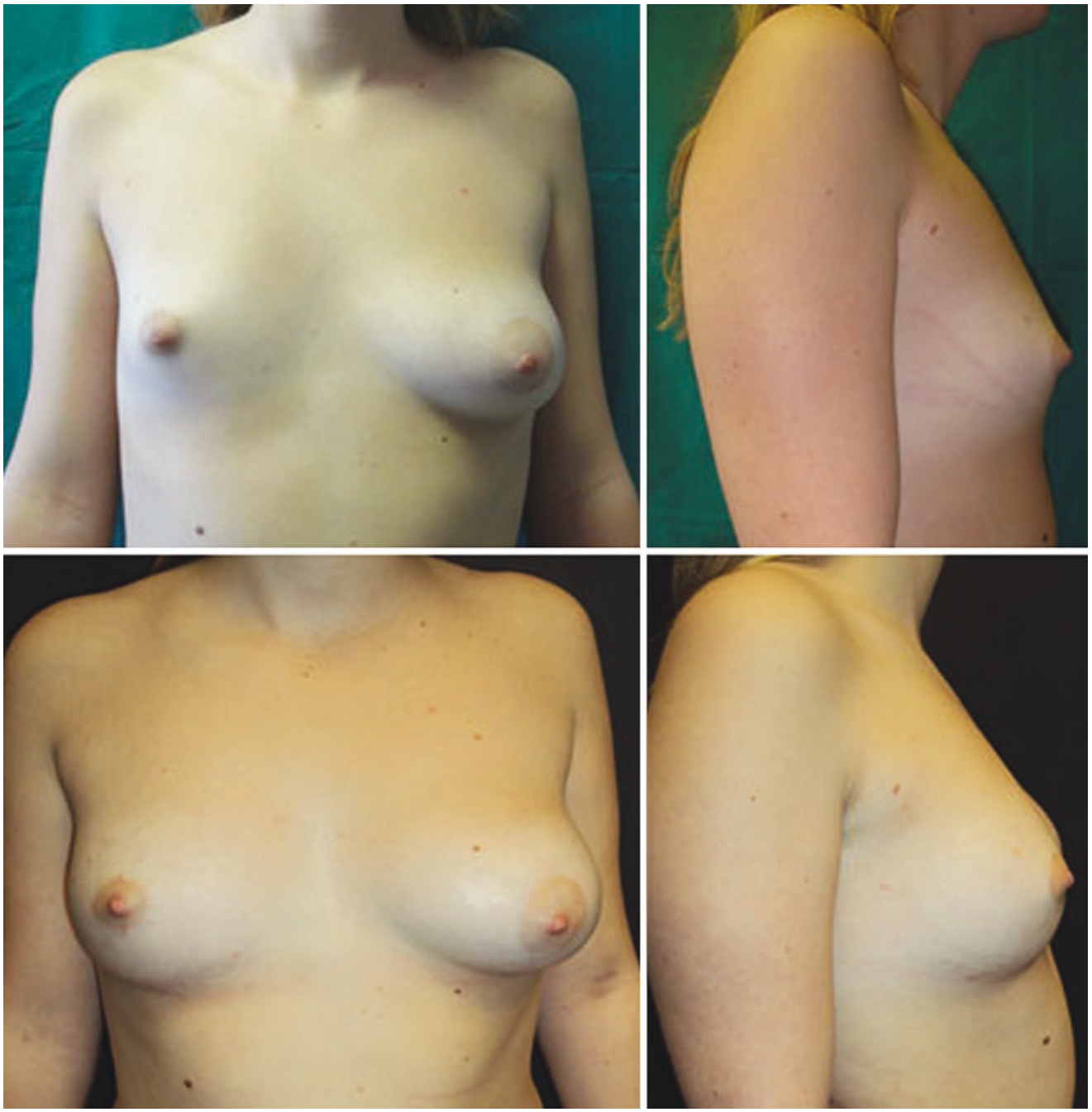

Fig. 6.7 Clinical Case: fat grafting for correction of congenital breast asymmetry. One year Post-op after three procedures

On the other hand, in vivo trials so far have not shown that CAL can increase the risk of breast cancer incidence or recurrence. Only one trial demonstrated the incidence of a single local malignancy, but further characterization showed that to be a secondary localization of pelvic metastasis which was independent from the treatment [43].

Since cancer occurrence is a long-term complication, there is still a lack of evidence about the oncological potential of CAL as those studies are still ongoing.

6.6 Conclusion

The identification of ASCs and their clinical application are quite recent concepts and it is very likely that in the coming years there will be a significant increase of clinical randomized trials on this subject that can hopefully bring a standardization of the technique. Currently, there is evidence that CAL is a useful and promising technique that in the future could lay the foundation for several clinical applications in regenerative surgery and breast surgery.

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