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A New Classification for Adipose-derived Stromal-cell Systems

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Summary: Obtaining regenerative cells from adipose tissue and their clinical use has become one of the most popular subjects of plastic surgery. However, there is no accepted classification in terms of methods. In this study, classification is proposed for the first time as a new idea. Accordingly, stromal cells can be obtained from adipose tissue by two approaches: direct methods for the bonds between parenchymal and stromal cells, and indirect methods, which target parenchymal cells rather than strong bonds, and increase the stromal cell ratio relatively. These methods can also be subclassified as fat (+), fat (−), fat (±) in terms of using the remaining fat in the final product as a graft. Direct methods include adinizing and enzymatic techniques; indirect methods include emulsification and micro-fragmentation/micronization techniques. In the enzymatic method, the fat tissue in the final product is considered dirty because it contains enzymes and must be discarded. That is why it is a fat (−) method. The adinizing method using ultra-sharp blades is fat (+) because the adipose tissue after the procedure can be used. Because the fat tissue is exposed to blunt pressure in emulsification techniques, it cannot be used as a graft. Thus, these are fat (−) methods. In micronization techniques using filter systems, there may still be intact adipocytes; therefore, it should be classified as fat (±). Adinizing provides both the highest efficiency and the full use of the end product. This classification will guide clinicians in terms of choosing the right product.
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INTRODUCTION

The purpose of all regenerative applications from fat is to expose the stromal cells. However, stromal cells in adipose tissue are connected to parenchymal cells, namely adipocytes, by very tight bonds and bridges.[1] There are only two ways to obtain these cells: either by directly targeting these ligaments and bridges and exposing the stromal cells; or indirectly, the proportion of stromal cells is increased, which is possible by eliminating or reducing the parenchymal cells.

The general approach focused on two different methods: enzymatic and mechanical.[2] In this study, a new classification was presented for obtaining stromal from adipose tissue using different criteria.

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NEW CLASSIFICATION

Direct Methods

Direct methods separate the stromal cells without killing the directly affected parenchymal cells by the bonds and bridges between parenchymal and stromal cells.

A. Fat (+) methods: Adinizing is a method where adipocyte and stromal cells are separated with ultra-sharp blades; the fat tissue can be used after the procedure and the ECM is preserved. Copcu and Oztan named the final product obtained in this method TOST (total stromal-cell).[3]
B. Fat (−) methods: These involve obtaining stromal cells via enzymatic methods. The final product is called SVF (stromal vascular fraction); the fat obtained during this process is not used.[2] It is considered waste because of the potential risk of enzymes being in it. Sese et al[4] showed that the main stromal cells are in the waste adipose tissue.

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The loss is about 90%, and almost 10 times as many cells can be obtained by mechanical methods.[3]

Indirect Methods

The aim in these methods is to remove the parenchymal cells completely or partially and to ensure that relatively more stromal cells remain in the final product.

A. Emulsification: Adipocytes, which are extremely sensitive to trauma, are emulsified by passing fat tissue between two syringes with the help of a nanofat connector[5]; stromal cells, which are more resistant to trauma, remain in the environment. The principle used here is Poiseuille law: The velocity of the steady flow of a fluid through a narrow tube varies directly as the pressure and the fourth power of the radius of the tube and inversely as the length of the tube and the coefficient of viscosity.[5] Because the blunt pressure created removes the parenchymal cells, they cannot be used for fat grafting.[6] So this method is fat (−).

B. Microfragmentation/micronization: The aim is to fragment and eliminate the parenchymal cells, or to decrease their efficiency and increase the relative stromal cell rate. For this purpose, filter,[7] bead,[8] centrifuge,[9] membrane systems,[10] and blunt pressure systems are used.[3] In fact, there is little or no amount of adipocyte spring in the final product. Although these can be fat (±) depending on the extent of the pressure applied in the procedure, there will definitely be some adipocyte death; so most of the adipocytes will never survive, as in adinizing (Figures 1–2 and Supplemental Digital Contents 1–6). (**See table, Supplemental Digital Content 1,** which shows the main view of new classification. **http://links.lww.com/PRSGO/C306.**) (**See figure 1, Supplemental Digital Content 2**, which shows the detailed explanations of new classification for the methods of obtaining stromal cells from adipose tissue. **http://links.lww.com/PRSGO/C307.**) (**See figure 2, Supplemental Digital Content 3**, which shows the appearance of tools used in all techniques in obtaining stromal cells from adipose tissue: enzymatic, mechanical [ultra-sharp blades, emulsification, and microfragmentation]). **http://links.lww.com/PRSGO/C308**. (See **Figure, Supplemental Digital Content 4**, schematic view of the ultra-sharp blade system and the adinizing method. The bridges and bonds are cut by ultrasharp blades without blunt pressure, and parenchymal cells [adipocytes] can be used after obtaining of stromal cells. **http://links.lww.com/PRSGO/C344**). (See **Figure, Supplemental Digital Content 5**, macroscopic views of the end product in all methods, **http://links.lww.com/PRSGO/C345**). See **Figure, Supplemental Digital Content 6**, A schematic view of the general principles of the ARAT [Adjustable Regenerative Adipose-tissue Transfer] approach described by Copcu and Oztan. A: Thicker adipocytes can be applied deep [eg, on the periosteum], whereas thinner ones can be applied to the surface. This approach allows for the application of fat grafts to different anatomical areas and different depths, just as in the application of hyaluronic acid. Thus, both more natural results are obtained, and visibility complications are prevented. MEST [Mechanical stromal-cell Transfer], on the other hand, can be applied to any area that requires regeneration. B: With ultra-sharp blades, the stromal cells, which are connected to the parenchyma with very tight bonds and bridges, are released, whereas stromal cells in quiescent mode are converted to regenerative mode. This should be considered as simultaneous cell-enriched lipotransfer. In other words, ultra-sharp blades not only allow adipocytes to reach the desired diameter, but also reveal their regenerative properties. **http://links.lww.com/PRSGO/C346**.)

Takeaways

Question: How should the techniques of obtaining regenerative cells from adipose tissue be classified?

Findings: There are two purposes in all methods: either the bonds and bridges between parenchymal and stromal cells are directly targeted and they are eliminated, or by applying blunt pressure or high force to the adipocytes, the stromal cell ratio is relatively increased, and the stromal cells are released. The use of adipocytes in the final product should be considered.

Meaning: This classification allows the clinician to choose the right path and scientists to evaluate existing products with a common approach. The highest efficiency is achieved in the adinizing technique and adipocytes can be used for grafting.

DISCUSSION

The aim in enzymatic methods is to release the stromal cells by dissolving the bonds between parenchymal and stromal cells such as collagenase or trypsin.[2] However, this process is considered biological drug production by authorities such as the FDA and EMA, and cGMP/GLP standards must be provided for it to be performed.[2] Enzymatic methods are much more expensive, more time-consuming and complicated. They require more equipment and staff than mechanical methods.[2] In addition, because the adipose tissue used in enzymatic methods is considered dirty, it must be discarded after the stromal cell is obtained. In mechanical methods, the aim is to cut the ligaments directly with ultra-sharp blades, which was defined as "adinizing" by Copcu et al.[11] Mechanical methods are classically defined as "emulsification" and "micronization/microfragmentation."[3] The popularization of mechanical methods occurred with the definition of "nanofat" by Tonnard in 2013, and it has become one of the most widely published topics in the literature in recent years.[5] However, although the term nanofat contains the word "fat," there are no viable adipocytes in the final

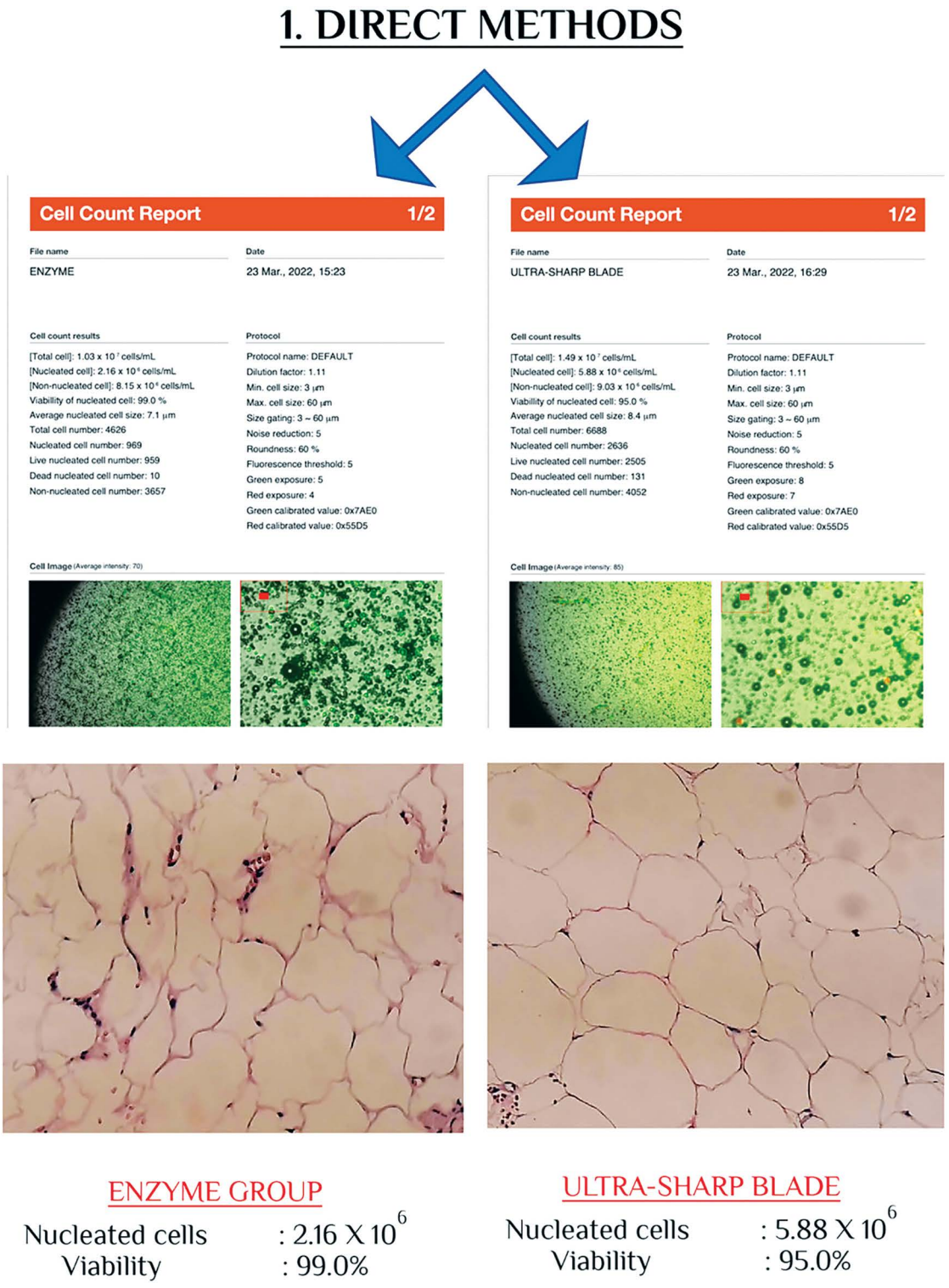

Fig. 1. Detailed view of the direct methods. Dual fluoroscopy results, histopathological microscopic images, nucleated cell count and cell viability are presented. 10 cc condensed fat was harvested from abdominoplasty specimen and used for enzyme and mechanical methods with ultra-sharp blades. Cell viability and count were made by dual fluoroscopy technique with acridine orange/propidium iodide stain. Histopathological assessment was made with hematoxylin eosin staining X 500 magnification. Results of each technique were presented with reports of dual fluoroscopy and microscopically. Cell amount and viability were also presented.

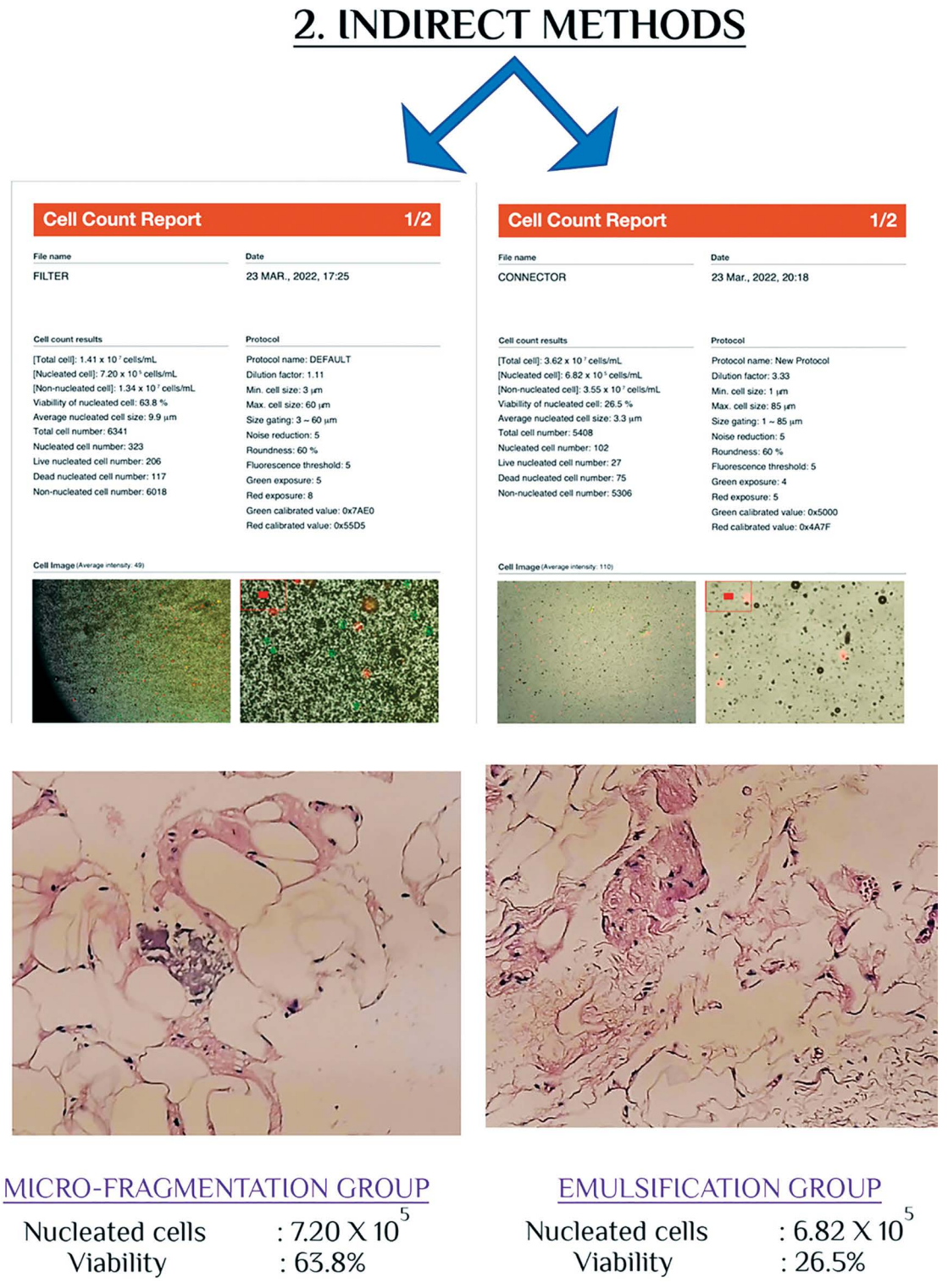

Fig. 2. Detailed view of the indirect methods. Dual fluoroscopy results, histopathological microscopic images, nucleated cell count and cell viability are presented. 10 cc condensed fat was harvested from abdominoplasty specimen and used for micro-fragmentation and emulsification techniques. Cell viability and count were made by dual fluoroscopy technique with acridine orange/propidium iodide stain. Histopathological assessment was made with hematoxylin eosin staining X 500 magnification. Results of each technique were presented with reports of dual fluoroscopy and microscopically. Cell amount and viability were also presented.

product.[5] The concept of micronization has a much longer history and was first used for drug administration in 1947.[12] This term was mostly used in adipose tissue instead of "microfragmentation," and the first use of this concept was by Carelli et al for a device in which stromal cells were obtained mechanically from adipose tissue.[13]

Copcu and Oztan have published a rather detailed review of mechanical methods.[3] In this study, they showed the evolution of mechanical methods and presented them in four steps. These are: Nanofat defined by Tonnard,[5] a "beads" system defined by Tremolada,[7] a "connector and filter system" defined by Cohen,[6] and, finally, ultra-sharp blade systems defined by Copcu and Öztan,[11] whose extensive literature review showed that the highest efficiency in terms of both cell number and viability is in the exact blade systems. They called the cutting of fat tissues with ultra-sharp blade systems "adinizing." This method is not microfragmentation because it directly targets the stromal and parenchymal intercellular bonds, and while obtaining stromal cells, the parenchymal cells are not damaged.

Copcu and Öztan described a new approach in fat grafting applications and named it adjustable regenerative adipose-tissue transfer.[11] In this approach, the aim is to apply different sizes of adipose tissue to different areas, as in hyaluronic acid filling applications. The authors have speculated that this approach allows the liberated stromal cells to switch from quiescent to regenerative mode, and provides the desired diameter in adipose tissue using an ultra-sharp blade system.[11] They named this approach simultaneous cell-enriched lipotransfer. It has been proved histopathologically that the integrity of the structure of the adipose tissue is preserved when the size of the adipose tissue is reduced from 4000 to 100 µm.[11] This approach will be highly advantageous in male and female genital applications, where a 400/600 µm diameter fat graft will be applied in thin-skinned and superficial areas, and at the same time significant skin improvement will be achieved with regeneration.[14] Similarly, while large-diameter fat grafting can be placed intramuscularly in breast tissue, applying a smaller diameter fat graft in areas closer to the surface will both increase the chance of success and reduce the risk of complications such as fat necrosis and microcalcification.[15] This approach applies to high-volume gluteal fat grafting. Preferring the desired fat size will not only create the desired result, but also allow for regeneration in the superficial area.[16]

In order for the fat graft to be successful in radiation injury and burn reconstruction, two basic problems must be solved. These are high pressure and vascular insufficiency.[17] The larger the fat graft, the higher the risk of compression. Low-diameter adipose tissue in this area will reduce the possibility of pressure. For vascular support, the liberation of stromal cells will increase regeneration or, in other words, angiogenesis, with cytokines.

CONCLUSIONS

Acceptance of this new classification will be a guide for surgeons. The viability of the adipose tissue as the final product has been shown, as well as the cell number and viability of the existing methods. Thus, if both regenerative application and fat grafting are planned in the same session, the right product will be selected.

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REFERENCES

1. Doi K, Tanaka S, Iida H, et al. Stromal vascular fraction isolated from lipoaspirates using an automated processing system: bench and bed analysis. *J Tissue Eng Regen Med.* 2013;7:864–870.
2. Aronowitz JA, Lockhart RA, Hakakian CS. Mechanical versus enzymatic isolation of stromal vascular fraction cells from adipose tissue. *Springerplus* 2015;4:713.
3. Copcu HE, Oztan S. Not Stromal Vascular Fraction (SVF) or Nanofat, but Total Stromal-Cells (TOST): a new definition. systemic review of mechanical stromal-cell extraction techniques. *Tissue Eng Regen Med.* 2021;18(1):25–36.
4. Sesé B, Sanmartín JM, Ortega B, et al. Nanofat cell aggregates: a nearly constitutive stromal cell inoculum for regenerative site-specific therapies. *Plast Reconstr Surg.* 2019;144:1079–1088.
5. Tonnard P, Verpaele A, Peeters G, et al. Nanofat grafting: basic research and clinical applications. *Plast Reconstr Surg.* 2013;132:1017–1026.
6. Stuzin JM. Discussion: Nanofat grafting: basic research and clinical applications. *Plast Reconstr Surg.* 2013;132:1027–1028.
7. Cohen SR, Tiryaki T, Womack HA, et al. Cellular optimization of nanofat: comparison of two nanofat processing devices in terms of cell count and viability. *Aesthet Surg J Open Forum.* 2014;1:ojz028.
8. Tremolada C, Colombo V, Ventura C. Adipose tissue and mesenchymal stem cells: state of the art and Lipogems technology development. *Curr Stem Cell Rep.* 2016;2:304–312.
9. Gentile P, Scioli MG, Bielli A, et al. Comparing different nanofat procedures on scars: role of the stromal vascular fraction and its clinical implications. *Regen Med.* 2017;12:939–952.
10. Hayashi S, Yagi R, Taniguchi S, et al. A novel method for processing adipose-derived stromal stem cells using a closed cell washing concentration device with a hollow fiber membrane module. *Biomed Microdevices.* 2021;23:3.
11. Copcu HE, Öztan S. New mechanical fat separation technique: adjustable regenerative adipose-tissue transfer (ARAT) and mechanical stromal cell transfer (MEST). *Aesthet Surg J Open Forum.* 2020;2:ojaa035.
12. Boyd EM, Dingwall RW. The absorption and elimination of sulfadiazine administered as tablets, as a gound (micronized) powder and as microcrystals. *Am J Med Sci.* 1947;213:549–556.
13. Carelli S, Messaggio F, Canazza A, et al. Characteristics and properties of mesenchymal stem cells derived from microfragmented adipose tissue. *Cell Transplant.* 2015;24:1233–1252.
14. Copcu HE. Indication-based protocols with different solutions for mechanical stromal-cell transfer. *Scars Burn Heal.* 2022;8:20595131211047830.
15. Delay E, Nachaoui H. Place des transferts graisseux dans les malformations des seins et du thorax [Lipomodeling for congenital breast deformities: Technique, results and indications]. *Ann Chir Plast Esthet.* 2022;67:319–334. . French.
16. Del Vecchio D, Kenkel JM. Practice advisory on gluteal fat grafting. *Aesthet Surg J.* 2022;42:1019–1029.
17. Klinger F, Murolo M, Siliprandi M, et al. Fat grafting in radiation-induced soft-tissue injury: a narrative review of the clinical evidence and implications for future studies. *Plast Reconstr Surg.* 2022;150:919e–920e.