LIPO-STEM DUO™

Morphology, MSCs viability and proliferation comparison of adipose tissue processed by different devices.

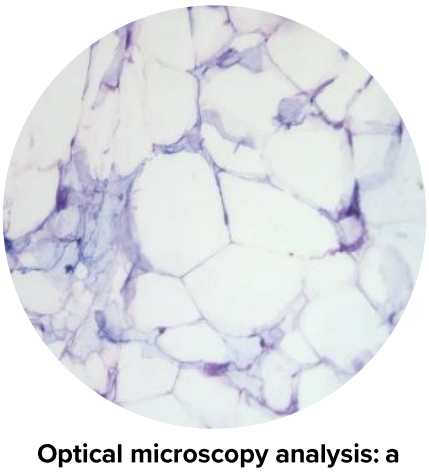
Preliminary Data from an International Public University Study

The abundance of MSCs in adipose tissue along with easy accessibility makes it an attractive source of MSCs for many clinical uses. Autologous microfragmented adipose tissue is a promising option due to its ability to release exosomes and promote tissue regeneration and is increasingly used in orthopaedic, plastic, and reconstructive surgeries.

The ability to accurately and efficiently preserve cell health is crucial to determine their regenerative potential.

Viability and proliferation are two distinct characteristics of cells: **viability** measures the number of living cells in a population, whereas **proliferation** measures cell division.
Both contribute to determining the regenerative potential of the autologous implant.

These preliminary data compare adipose tissue processed with different devices and show the impact of different methods on **cell prosperity and health.**



Optical microscopy analysis: a portion of the adipose sample of LIPO-STEM DUO™.

All samples have been evaluated at a morphological level by using the whole-mount assay.
The emulsion has been swiped in a histological glass and stained with Toluidine Blue (Sigma-Aldrich, Milan, Italy). All slides are going to be examined under an Olympus BX-51 microscope (Olympus, Tokyo, Japan) equipped with a digital camera (DKY-F58 CCD JVC, Yokohama, Japan).

Methodology:

- **Adipose tissue enzymatic digestion**

The remaining portion of adipose tissue has been digested according to the standard laboratory protocol using collagenase type I. The fat digestion has been done under agitation at 37°C for 45 min, and subsequently, the enzymatic action has been blocked with a complete culture medium (DMEM supplemented with 10% FBS (Foetal Bovine Serum), 1% 1:1 P/S (Penicillin/Streptomycin) and 0.6% Amphotericin B) followed by centrifugation at 7000 rpm for 5 min. The formed pellet has been resuspended in 1X lysis buffer for 10 min, filtered and re-centrifuged to obtain the stem cell pellet. The cells will be seeded in a T25 flask for subsequent analysis.

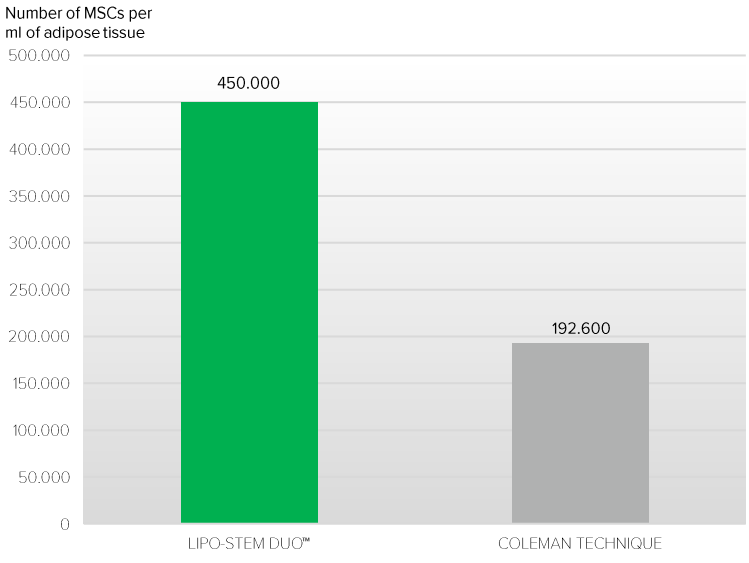
- **Cellular yield**

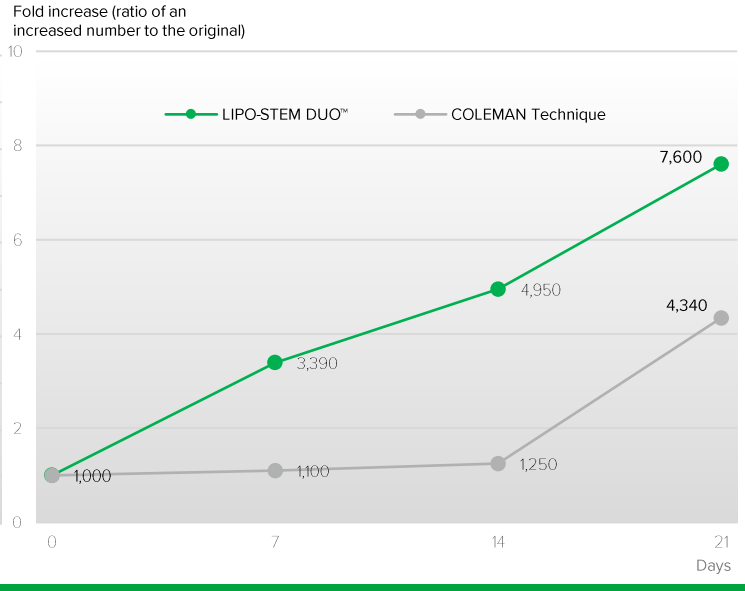
The extracted cells have been counted for cellular yield calculation considering the number of extracted free cells divided by the processed volume of fat. The number of living cells has been calculated using the Trypan Blue exclusion assay in a CytoSMART counter (Automated Image-Based Cell Counter, version 1.5.0.16380, CytoSMART Technologies B.V., Eindhoven, Netherlands).

- **Proliferation capacity**

The extracted cells have been seeded on a 25 cm^2 T-flask with a complete culture medium and incubated in a humidified atmosphere at 37°C with 5% CO_2. The first medium change has been performed after 72 h from the enzymatic digestion and the subsequent changes every 48 h. The proliferation capacity is determined considering the required days to reach 80% confluence.

Remark: reported preliminary data already show trends but they are not statistical yet.



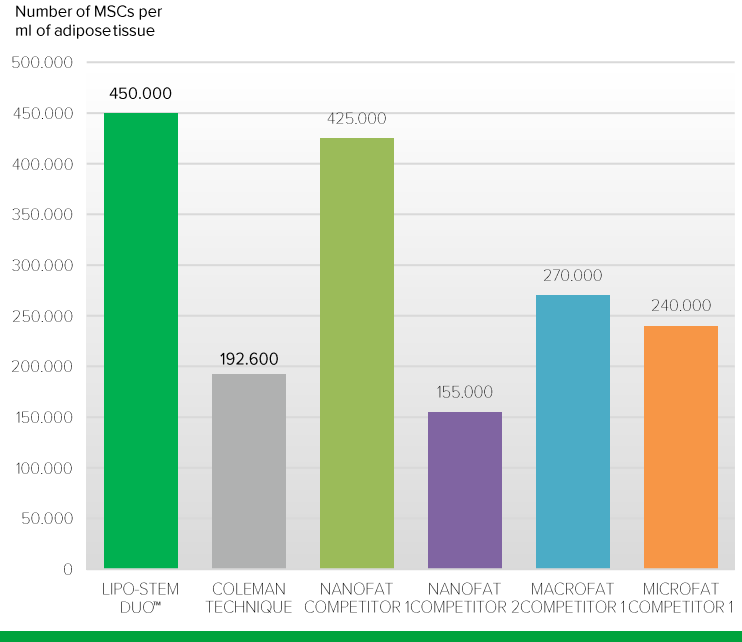


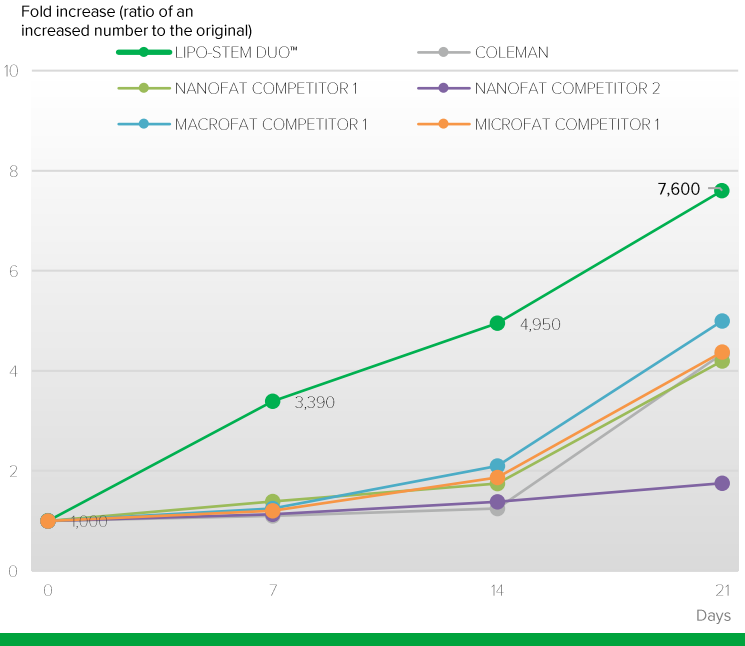
Cell viability after harvest - gentle washing vs centrifugation

The graphic shows a focused comparison between the gentle washing method of LIPO-STEM DUO™ and the Coleman Technique (centrifugation). It is clearly visible how the number of harvested MSCs per ml of adipose tissue with gentle washing outperforms the centrifugation method of more than 2 times.

Cell proliferation in vitro after 21 days:

In vitro, MSCs cells of LIPO-STEM DUO™ proliferate growing more than 7 times in 21 days, while MSCs harvested with the Coleman Technique (centrifugation) grew only 4 times. The graphic witnesses the exceptional vitality and health status of cells processed with the gentle washing method.as opposed to the centrifugation method.





Cell viability after harvest - comparison of all samples

The harvesting method has a great impact on cell survival, safeguarding or discouraging **MSCs' survival.** The count of cellular yield and the comparison among different devices shows how LIPO-STEM DUO™ outperforms all other methods. The second best result was found with NANOFAT COMPETITOR 1 (but later the sample did not show an equally good proliferation). The worst result has been found with NANOFAT COMPETITOR 2.
Harvesting methods affect the number of live cells and determine the regenerative potential of the adipose tissue.

Cell proliferation in 21 days - comparison of all samples

The harvesting method definitely has an impact also on the successive **proliferation of cells**. Shocked or traumatized cells reveal a significantly lower capacity for proliferation, affecting their successive capacity for tissue regeneration. The graphic shows how the gentle washing method of LIPO-STEM DUO™ outdoes all the others almost doubling all of them. The worst performance of proliferation is given by NANOFAT COMPETITOR 2, which was also the method that returned the worst result of harvesting.

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Technical Documentation Center - Biopsybell S.r.l. Società Unipersonale
Via Aldo Manuzio 24 - 41037 Mirandola (MO)
Tel. 0585 27850
www.biopsybell.com