Bologna, September 21st, 2023

Hereby reported are the results concerning the viability and structural analyses performed in human fat tissue derivatives that had been obtained following a lipoaspirate processing by the aid of the LIPOSMART device and method (https://liposmart.trade).

The analyses have been executed at the *National Laboratory of Molecular Biology and Stem Cell Engineering*, of the "*Istituto Nazionale di Biostrutture e Biosistemi*" (INBB – www.inbb.it), located in via di Corticella 183, 40128 Bologna, Italy. I'm the Scientific Director of this Laboratory, and the Chief of the *Division of Bologna* of INBB, encompassing the INBB Research Units of Bologna, Firenze, and Siena, Italy.

We have used a Nikon inverted Microscope Eclipse Ti-E (Nikon Instruments, Tokyo, Japan), equipped with a DS-03 Digital Sight camera connected to a computer, and handled through a version 4.30 of the NIS-Elements software (Nikon Instruments).

RISULTS

CELL VIABILITY

The cell vitality assay (Figure 1) has been conducted by the aid of a peculiar application we have developed of the *LIVE/DEAD™ Viability/Cytotoxicity Kit, for mammalian cells* https://www.thermofisher.com/order/catalog/product/L3224#/L3224

Vitality\Mortality

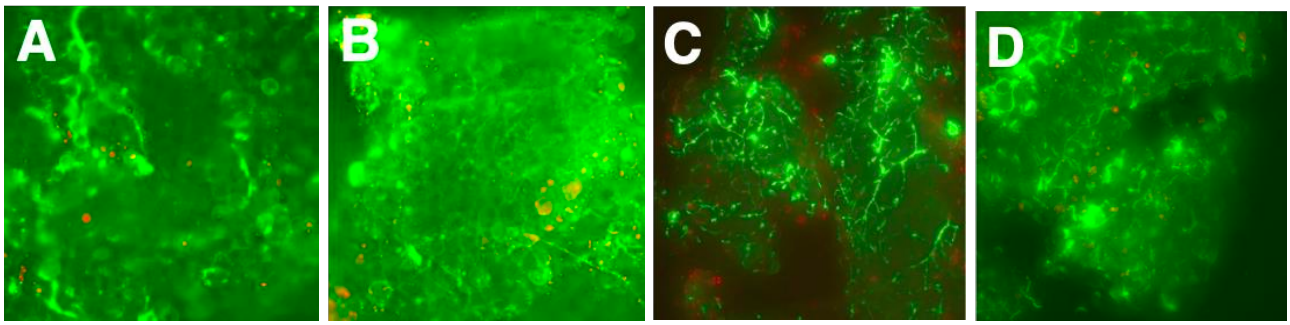


Figure 1

Cell Vitality. Representative images: A, C: original lipoaspirates. B, D: their corresponding Liposmart products. The figure shows the remarkable presence of intact cellular contours (stained green), indicating a cell surface integrity. The number of cells exhibiting a red staining, resulting from the internalization of propidium iodide through a damaged plasma membrane is extremely low. Noteworthy, the number of red-stained cells resulted to decrease in the Liposmart product, as compared with its corresponding original lipoaspirate, indicating that the fat processing achieved by the device was capable of getting rid of suffering cells, thus increasing the overall yield off viable elements. The Liposmart samples also showed consistent, well-evident alignments of green-stained, viable cells, which are referrable to as vascular structures within the sample itself.

STRUCTURAL ANALYSIS OF THE STROMAL-VASCULAR NICHE

On aliquots of the same samples utilized for the viability assays, we have also analyzed the structure of the *stromal-vascular niche*. This is a “nano-topography”, where cells with pericyte identity (now emerging as the true precursors for mesenchymal stem cells) appear as perivascular elements within slit-like capillaries wedged between pre-adipocytes and stromal stalks. The *stromal-vascular niche* is a *place* where physical and chemical signaling coalesce to shape the biological features of tissue-resident stem cells, specifying both their differentiating and paracrine/autocrine repertoires.

The samples have been fixed and incubated with monoclonal antibodies targeted against VE-Cadherin (green staining) and CD31(red staining) (Figure 2). VE-Cadherin is an essential adhesion molecule in creating cell-to-cell physical and functional communications. It is also a remarkable component of the integrity and permeability of the vascular endothelium. CD31 (PECAM-1) is a pivotal marker for endothelial cells and pericytes, being also used to locate these cells and trace their distribution pattern within biological samples. Together, these markers allow a fine structural characterization of the *stromal-vascular niche*.

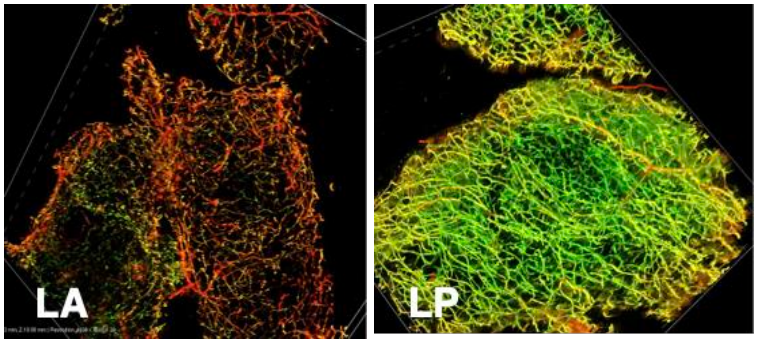


Figure 2.
LA: original lipoaspirate. LP: the corresponding Liposmart product. These representative images show the remarkable texture of the *stromal-vascular niche*, as depicted by VE-Cadherin (green) and CD31 (red) staining. Intriguingly, the vascular architecture appeared more preserved in the Liposmart product, than in its original lipoaspirate. This finding indicates that the Liposmart method and device were capable to afford the recovery of an optimal architecture of the *niche* following the structural remodeling imposed by the stress of the liposuction procedure.

Figure 3 shows a whole-field view of the *stromal-vascular niche*, further confirming these findings and conclusions. For the fluorescence analyses the same microscopy apparatus and set-up described above have been used.

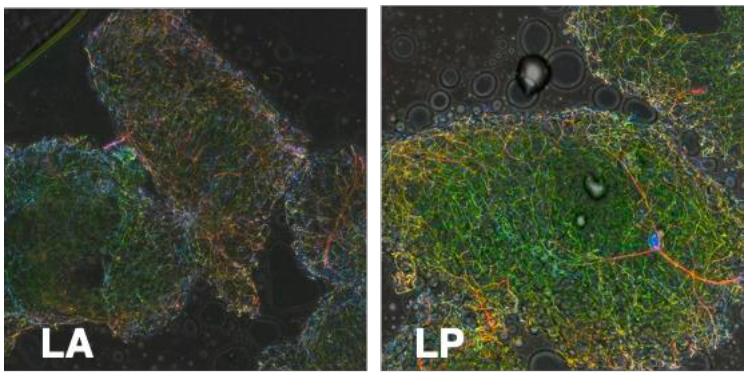


Figure 3.
A whole-field view of VE-Cadherin (green) and CD31 (red) staining in the original lipoaspirate (LA) and in its corresponding Liposmart product (LP) confirms the structural recovery and improvement in the architectural organization of the *stromal-vascular niche* in the LP, when compared to LA. The profile of the red staining for CD-31 is very suggestive, highlighting how the pericytic elements (perivascularly located) follow the vascular network and intertwine with its path.

In additional characterization analyses, we investigated the distribution pattern of perilipin and CD68 in both lipoaspirates, and their corresponding Liposmart products (Figure 4). Perilipin (green staining) is a marker for the structural and functional integrity of prediapocytes and adipocytes, two important cellular elements in the modulation of the paracrine features of the *stromal-vascular niche*. CD68 (red staining) is a marker for macrophages, a cell type that is increasingly emerging as a major player in regenerative processes. After tissue injury, these cells undergo marked phenotypic and functional changes to play critical roles during the initiation, maintenance, and resolution phases of tissue repair.

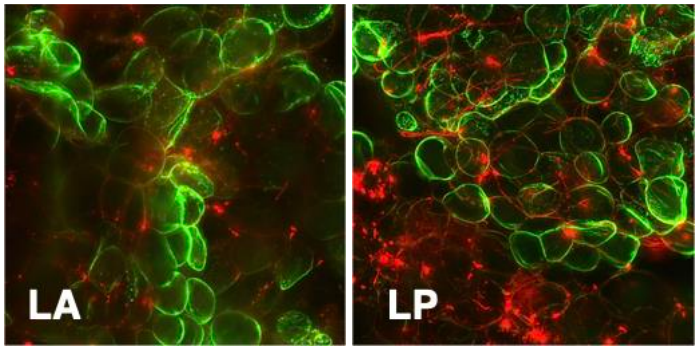


Figure 4.
LA: original lipoaspirate. LP: the corresponding Liposmart product. The Liposmart product exhibited a more clear/sharp contour in the membranes of pre-adipocytic elements (green), and macrophages (red), with a more ordered architectural organization of these cells, when compared to the original lipoaspirate. Consonant with the images in Figure 2, these observations again suggest that the tissue processing through the Liposmart method and device prompted a significant recovery in essential cellular and architectural features of the processed samples.

CONCLUSIONS

From my point of view, I believe that the Liposmart technology represents a new paradigm in the processing of adipose tissue, with unprecedented perspectives for the most varied applications in Regenerative Medicine, as well as Aesthetic and Reconstructive Medicine.

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