LIPO-STEM DUO

PRODUCT CHARACTERIZATION

PERFORMED BY

PROF. CARLO VENTURA

Director of the Laboratory of Molecular Biology and
Stem Cell Bioengineering of the National Institute of
Biostructures and Biosystems (INBB - www.inbb.it)

bps medica™

www.biopsybell.com

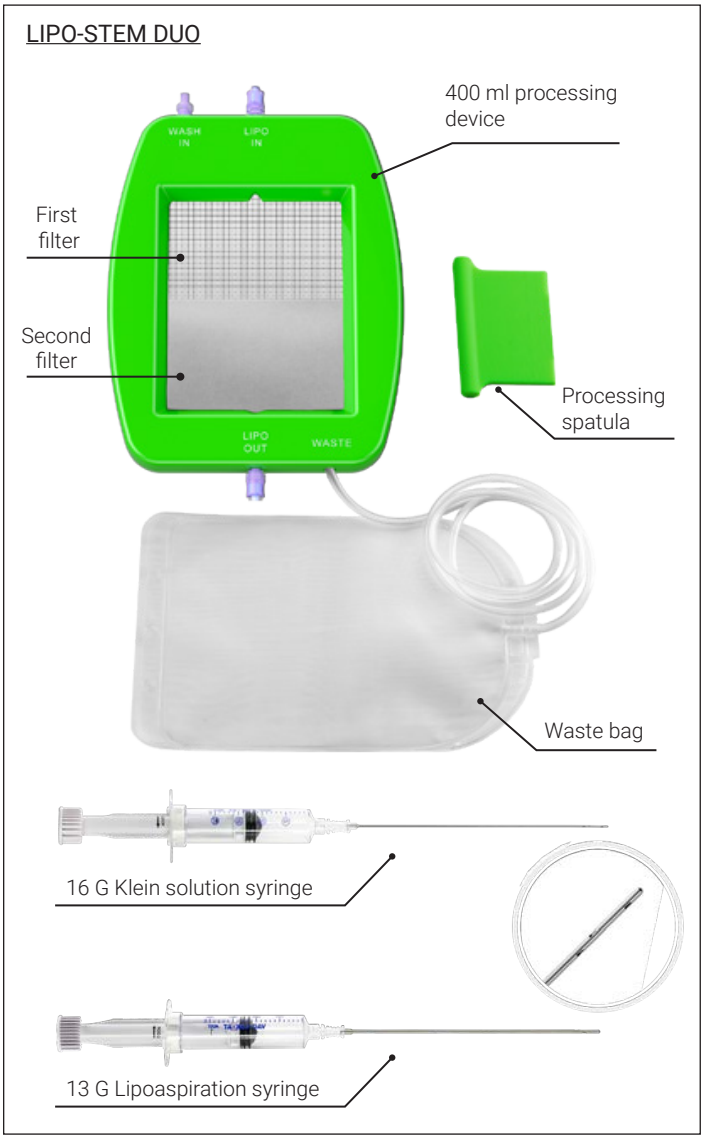
Introduction

LIPO-STEM DUO is a closed-circuit single-use kit for adipose tissue microfragmentation and purification without any centrifuge and with a minimal manipulation.

The filtering system consistently microfragments the adipose tissue while manteining its biological properties and maximizing the regenerative potential. The entire processing phase of the liposuctioned tissue occurs inside the device thanks to continuous saline solution washing. This allows to reduce the cellular stress eliminating any traumatic action that may damage the extracellular matrix and its essential trophic and anti-inflammatory function.
The collection and processing bag is equipped with two filters:

- the first filter microfragments the adipose tissue, while retaining the eventual fibrotic tissue
- the second filter with denser mesh retains the reduced (microfragmented) adipose tissue that is washed with saline solution eliminating all the oily and blood residues which might cause inflammation of the treated tissues.

The final microfragmented and purified product is an autologous adipose tissue that keeps the biological properties of the original tissue intact and can easily be injected even through very thin needles.



The following report shows the results of a **comparative structural and viability analysis** between **LIPO-STEM DUO** device, which features a double filtration processes, and a different processing device with a single filtration process.
The test was carried out on human adipose tissue preparations obtained from the same subject: the viability/mortality analysis was performed immediately upon receipt of samples, then both samples were fixed and incubated with monoclonal antibodies directed to VE-Cadherin (red staining) and CD31 (green staining). In the following days, we conducted an analysis of the vascular-stromal niche structure on both samples.

The analysis were conducted by **Prof. Carlo Ventura** at the National Laboratory of Molecular Biology and Stem Cell Bioengineering of the National Institute of Biostructures and Biosystems (INBB), at the Innovation Accelerators of the CNR Research Area in Bologna. A Nikon Inverted Microscope Eclipse Ti-E (Nikon Instruments, Tokyo, Japan) equipped with a DS-03 Digital Sight camera connected to a computer and managed through NIS-Elements imaging software, version 4.30 (Nikon Instruments), was used.

Viability and Mortality analysis

Viability \ Mortality

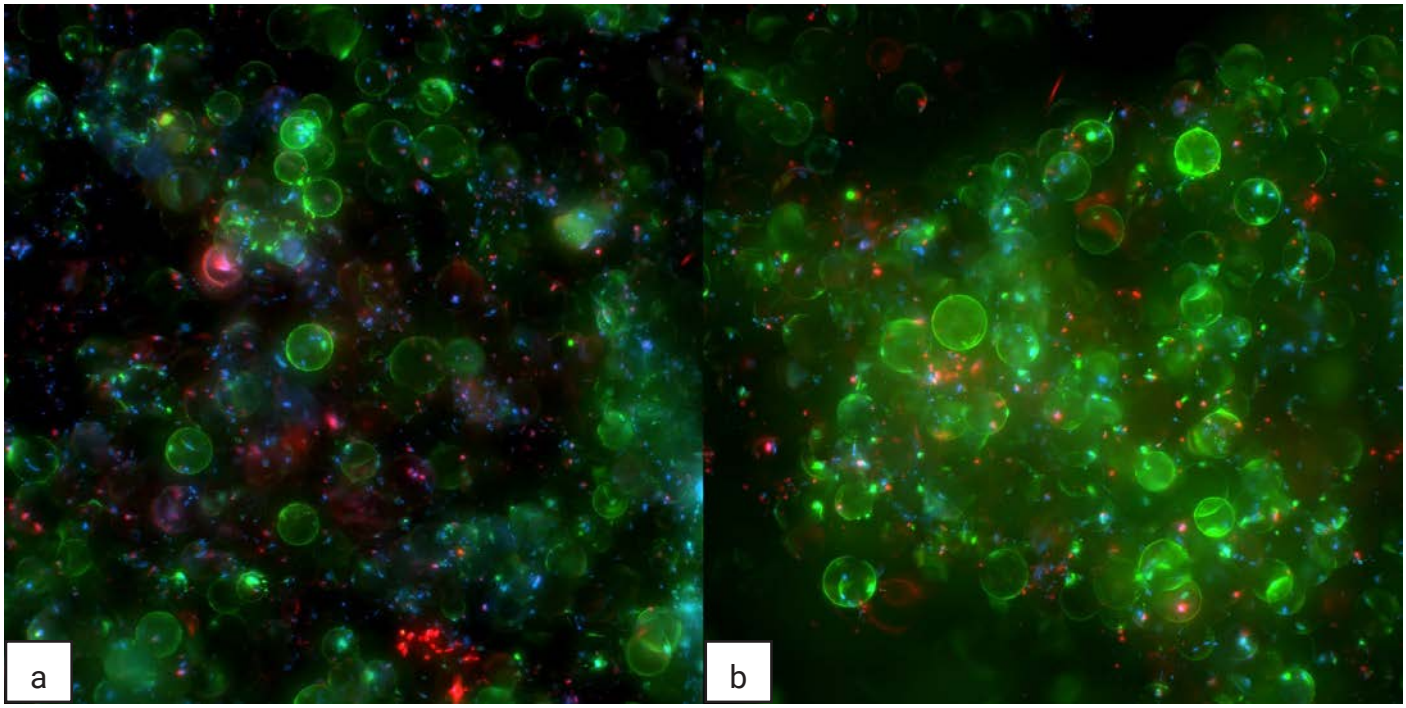


Figure 1 - Tissue Derived from Single Filtration (a), Tissue Derived from Double Filtration (using LIPO-STEM DUO device) (b)

Results

The tissue derivative obtained by single filtration (left panel), shows a considerable impairment of cell viability: the nuclei in which the propidium iodide (indeed in red) has succeeded in penetrating a non-intact membrane and reaching the DNA can be seen in red in Figure 1.
On the other hand, in the tissue derivative obtained by double filtration (right panel), we note the extensive presence of the intact membrane rim (green staining) of the adipocyte cells (round and large), which reflects the quality of preservation of the sample.
The adipocytes are in fact “stretched” and the membrane is very thin, unlike other cells that are more compact. Therefore, any damage occurring while processing the lipoaspirate would cause the adipocytes to readily lose membrane integrity. The number of cells showing a red staining relating to the passage of propidium iodide through a damaged membrane is, in this case, significantly lower than in the product obtained by single filtration. Moreover, only the sample obtained by double filtration shows consistent alignments of cells with green staining, and therefore intact, which seem to outline probable vascular structures within the sample itself. Such alignments are not seen in the sample obtained using single filtration.
With regard to the sample obtained by double filtration in particular, the possibility that at least some of the cells stained in red are still viable cannot be ruled out. In this respect, it is possible that the dye may pass through microholes in still viable cells in morphofunctional recovery.
This eventuality should not be postulated in the case of the sample obtained by single filtration, since this sample shows a pronounced structural disruption and a minimal density of green-stained cells.

Vascular-stromal niche structure analysis

Introduction

The results of an analysis of the structure of the vascular-stromal niche carried out on human adipose tissue preparations obtained using two different processing methods, one with a single filtration device and the other using **LIPO-STEM DUO** device with double filtration processes, performed on lipoaspirate taken from the same donor, are shown below.
The vascular-stromal niche can be defined as a "nano-topography" in which pericyte components (now believed to be the true precursors of Mesenchymal Stromal Cells - MSCs) are found in the perivascular environment in the context of capillaries with a distinctive architecture, interspersed between cell components with pre-adipocyte/adipocyte features. The vascular-stromal niche therefore represents the "site" where physical and chemical signals are integrated to determine the biological characteristics of the stem cells residing there, both in terms of differentiation capacity and the secretion of molecules with paracrine and autocrine activity.

Both samples were fixed and incubated with monoclonal antibodies directed to VE-Cadherin (red staining) and CD31 (green staining). VE-Cadherin is an adhesion molecule, very important in maintaining the integrity and permeability of the vascular endothelium. CD31 (PECAM-1) is an essential marker of endothelial and pericyte cells, and is used to detect these cells and trace their distribution. These markings enable us to characterise the fine structure of the vascular-stromal niche.

Results

In the images representing the analysis carried out we can appreciate how the architecture of the vascular-stromal niche is severely damaged and disrupted in the sample obtained by the single filtration process. This can clearly be seen in two distinct fields of analysis, shown below in Figure 2.

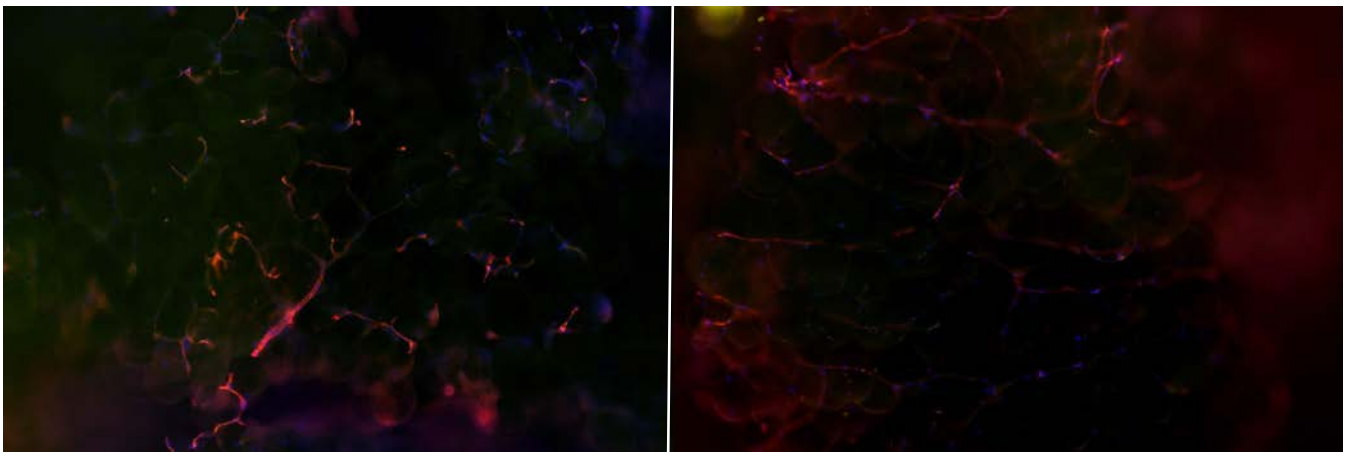


Figure 2a

Figure 2b

The vascular pattern is very difficult to trace. There are no clear relationships between vascular structures, which do not "outline" specific geometries, as can be seen from the trend of the VE-Cadherin marking (red staining). The presence of endothelial and pericyte components is also difficult to detect (the green marking relating to the expression of CD-31 is very faint, and does not outline precise architectures in this case). Adipocyte components are also very rare and difficult to detect.

By contrast, the architecture of the vascular-stromal niche is very well preserved in the sample obtained by the double filtration process with **LIPO-STEM DUO**. This is clearly visible in Figures 3 and 4, shown below.

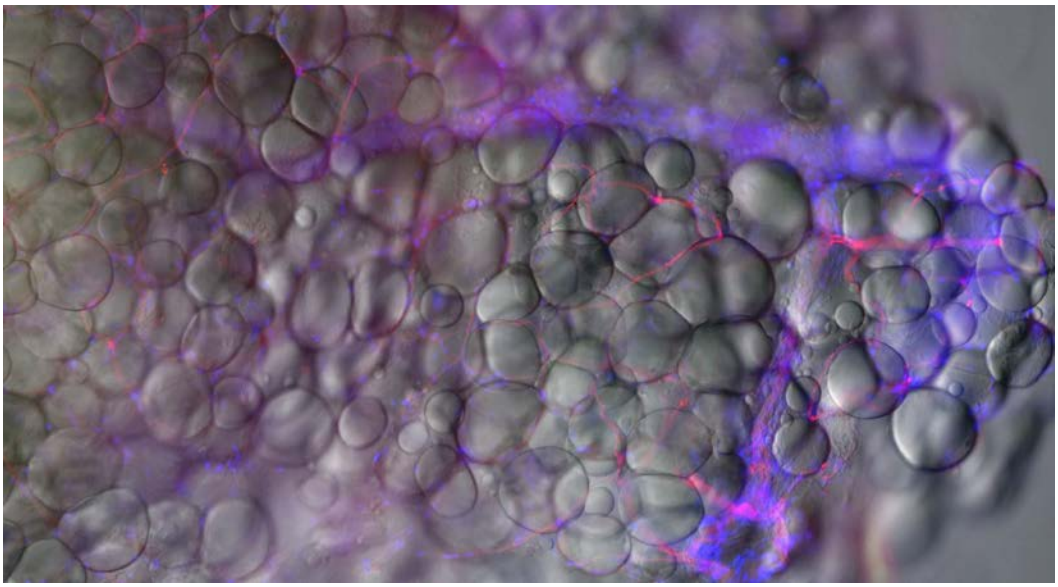


Figure 3

Figure 3 shows the perfect structural integrity of the adipocyte and preadipocyte components (large, round cells: the preadipocytes are seen with a smaller diameter). The red marking (VE-Cadherin), which demonstrates the presence of vascular reticulum, is very prominent. The blue marking is related to DAPI, a "cell-permeant" molecule that attaches itself to DNA and thus provides an indication of the remarkable "cellularity" associated with the vascular reticulum marked in red.

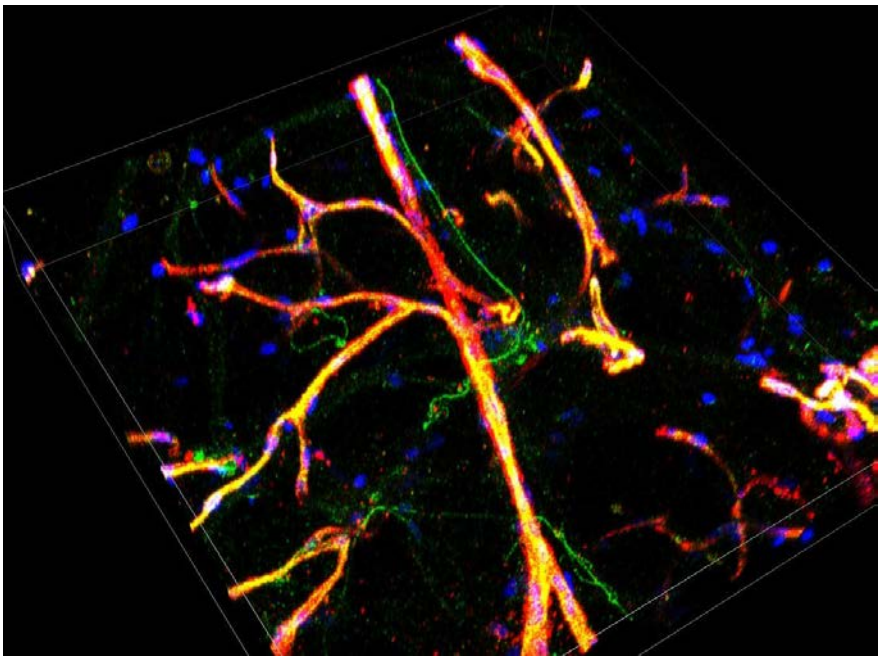


Figure 4

Figure 4 shows a multi-layer reconstruction of the vascular reticulum and pericyte components of the vascular-stromal niche in the sample obtained by the double filtration process. The image unequivocally documents how the vascular architectures (red marking with VE-Cadherin) are perfectly preserved; their geometry has very clear, well-defined contours. The profile of the green marking in relation to CD-31 is very suggestive, and shows how the pericyte components (with perivascular localisation) follow and intertwine with the pathway of the vascular reticulum.

Conclusions

Conclusions:

From Prof. Carlo Ventura point of view, **LIPO-STEM DUO** technology, manufactured by Biopsybell S.r.l, represents a new concept in the processing of adipose tissue, with extremely interesting prospects for the most varied applications in Regenerative Medicine and Aesthetic and Reconstructive Medicine.

TECHNICAL REPORT PERFORMED BY

PROF. CARLO VENTURA

Director of the Laboratory of Molecular Biology and Stem Cell Bioengineering of the National Institute of Biostructures and Biosystems (INBB - www.inbb.it) at the Innovation Accelerators of the CNR Research Area in Bologna.

Our company

BPB MEDICA™ is an Italian manufacturing company specialized in the design, production and marketing of high qualitative healthcare products for medical use and medical-surgery devices.

BPB MEDICA™ was founded in 1999 by the Bellini family, boasting thirty year's experience in the biomedical sector. The founder, Carlo Bellini Sr., started the business in 1968 and has passed down to his heirs ethics, integrity and spirit of sacrifice. Today BPB MEDICA™ has leveraged its 50 years experience to develop new innovative product lines, growing the company on international level.

50 YEARS EXPERIENCE

1968 — MEDICAL BUSINESS BEGINNING: The founder, Carlo Bellini Sr, started his business in the medical field, founding some of the most important companies of our medical district

1999 — BIOPSYBELL S.R.L. FUNDATION: Carlo Bellini Sr's daughter founded Biopsybell srl: "Biopsy" as the initial core business and "Bell" as Bellini family name.

2008 — THIRD GENERATION OF THE FAMILY: Carlo Bellini, founder's nephew, joined the company and focused on export sales development.

2012 — THE EARTHQUAKE: The company was damaged by an heavy earthquake: the family worked hard to keep the production open. After only 2 weeks from the EQ was back in business.

2014 — SPINE SURGERY: Development and launch of Spine product line.

2015 — EXPORT EXPANSION: Export success: 41 foreign countries served (35%TNR).

2016 — NEW SITE: The intensive growth of the last few years required a new and bigger production site.

2018 — A.R.T. LINE: Development and launch of Assisted Reproduction product line.

2019 — EXPORT ORIENTED: 70 countries served (70% TNR).

2019 — REGENERATIVE & ORTHO-BIOLOGICS: Development and launch of Regenerative Medicine & Ortho-Biologics product line.

2020 — AESTHETIC LINE: New production line for Aesthetic and Reconstructive surgery.

Our company

BPB MEDICA's™ philosophy is to grow alongside the needs of patients, doctors and hospital staff in general. Backed by the experience acquired by the company's specialized technical personnel and thanks to newly-adopted technologies, BPB MEDICA™ has quickly managed to make a name for itself on the domestic and international markets.

70

COUNTRIES SERVED

BPB MEDICA™ provides painstaking service to its clientele and its primary aim is product quality. The **internal Regulatory and Quality Departments** conducts rigorous tests, from the raw materials to the equipment and the finished product. This allowed the company to obtain CE, ISO 13485 and the establishment registration by FDA.

BPB MEDICA™ operates with high qualitative standards aimed at increasing customer satisfaction through a continuous improvement regime. We conduct multiple quality tests during the entire production process: this allows to reduce the production waste and grant high quality products.

Thanks to the **internal R&D Department** BPB MEDICA™ conducts constant research in the reference pathologies with an aim to ever better qualifying and improving its production standards and aiding the development of new products.

OUR PRODUCT LINES

- SPINE SURGERY
- ORTHO-BIOLOGICS
- ASSISTED REPRODUCTION
- BIOPSY
- INTENSIVE CARE
- AESTHETIC

CLEAN ROOM
Manual & automatic assembly, packaging.

METAL REFINISHING DEPARTMENT
Cutting, grinding, sharpening, cleaning, echogenic marking, sealing, reduction.

MOULDING DEPARTMENT
Vertical and horizontal molding.

OUR SERVICES

QUALITY & REGULATORY DEP.
Our primary aim is product quality and provide painstaking service. Our regulatory and quality teams conduct rigorous tests for this purpose.

OEM & PRIVATE LABEL SERVICES
A la cart production, with customer's brand name and custom color. Full-service support of Regulatory and Quality documentations.

RESEARCH & DEVELOPMENT
Constant research in order to increase production and quality standards while developing new products.

MARKETING SUPPORT
Video tutorial, case report, presence at the major medical congresses, organization of training and courses.

PRODUCTION PROCESS CONTROL
Complete manufacturing process carried out internally, from design to final packaging.

PERMANENT STOCK
Top selling items are always available in stock and can be shipped within 24 hours.

Contact us for further information:

BIOPSYBELL S.R.L.
Via Aldo Manuzio 24
41037 Mirandola – MO – ITALY
T. +39 0535 27850 – F. +39 0535 33526
C.F./P.Iva 02615000367

BPB MEDICA

www.biopsybell.com

international1@biopsybell.it

DTQMWSV00EN - Rev.00 22/01/2020