LIPO-STEM DUO™

PRODUCT CHARACTERIZATION

PERFORMED BY

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WASH
IN
LIPO
IN
LIPO
OUT
WASTE

Adipose Tissue MSCs: introduction

In the past few years, interest in adipose tissue as an ideal source of mesenchymal stem cells (MSCs) has increased. These cells are multipotent and may differentiate in vitro into several cellular lineages, such as adipocytes, chondrocytes, osteoblasts, and myoblasts.
The idea that fat tissue is an optimal source of MSCs is supported by the abundance of these cells in his tissue compared with other tissues, such as the widely used bone marrow, and by their easy access (Bianchi et al. 2013, Heinburg et al. 2004, Strem et al. 2007). Indeed, 1 in 100 adipose tissue cells is an MSC, compared with 1 in 100,000 bone marrow cells.
In addition, adipose-tissue-derived MSCs, secrete a variety of bioactive molecules that act in a paracrine fashion to prime and sustain angiogenic, antifibrotic, antiapoptotic, and immunomodulatory responses in the target tissue (Caplan 2007, Caplan-Dennis 2006).
MSCs from adipose tissue, are being used increasingly for many clinical applications, such as orthopaedic, plastic, and reconstructive surgery. Adipose-derived MSCs are routinely obtained enzymatically from fat lipoaspirate as SVF and/or may undergo prolonged ex vivo expansion, with significant senescence and a decrease in multipotency, leading to unsatisfactory clinical results.
Early methods involved the use of cell cultures, with proliferation and subsequent differentiation of mesenchymal cells (MSCs). However, in reality, there are many disadvantages related to the cost and complexity of managing these procedures, now considered advanced cell therapies.
Consequently, the possibility of using MSCs autologously, through minimal manipulation directly in operating theatres, is of great interest.
MSCs from adipose tissue (ADSCs) can be used in at least three ways. The first and only option with which one can properly speak of ADSC is in vitro culture and possible expansion. However, this option does not apply to clinical practice, except through Good Manufacturing Practice procedures that make the cells a drug. The second alternative is an enzymatic digestion of adipose tissue, which delivers, into the hands of the operator, a heterogeneous population of cells containing, in addition to MSCs, endothelial cells, leukocytes and pre-adipocytes. This by-product of enzymatic digestion is known as a vascular-stromal fraction (SVF).
Lastly, the autologous use of ADSCs can be performed in operating theatres through the microfragmentation of adipose tissue (μFAT, microfat). Whereas SVF is a heterogeneous population of cells separated from each other and falls under the concept of dose-effectiveness, μFAT is a true minimally manipulated tissue transplantation that relies more on cell quality than cell quantity.
In addition to ensuring greater permanence at the implant site due to the viability of the tissue itself, the procedure for obtaining μFAT is able to preserve the cell's ability to release exosomes, whereas enzymatic digestion eliminates this possibility (García-Contreras et al., 2014). Exosomes are nano-sized particles released by cells, consisting of a phospholipid bilayer with numerous messengers inside, including various proteins, as well as miRNA and DNA. Cell-cell communication via exosomes, rather than just the simple release of proteins, allows cell regulation on several levels, on transcription (DNA), translation (miRNA) and cell signalling (proteins), all in a way that is responsive to stimuli from the surrounding microenvironment.
MSCs from micro-fragmented adipose tissue, in addition to differentiating, are able to exert a paracrine effect by releasing growth factors, triggering tissue regeneration through a direct trophic stimulus on the tissue (Murphy et al., 2013) - (Caplan, 2008) and promoting angiogenesis (Boomsma et al., 2012), progenitor recall and a reduction in inflammation.

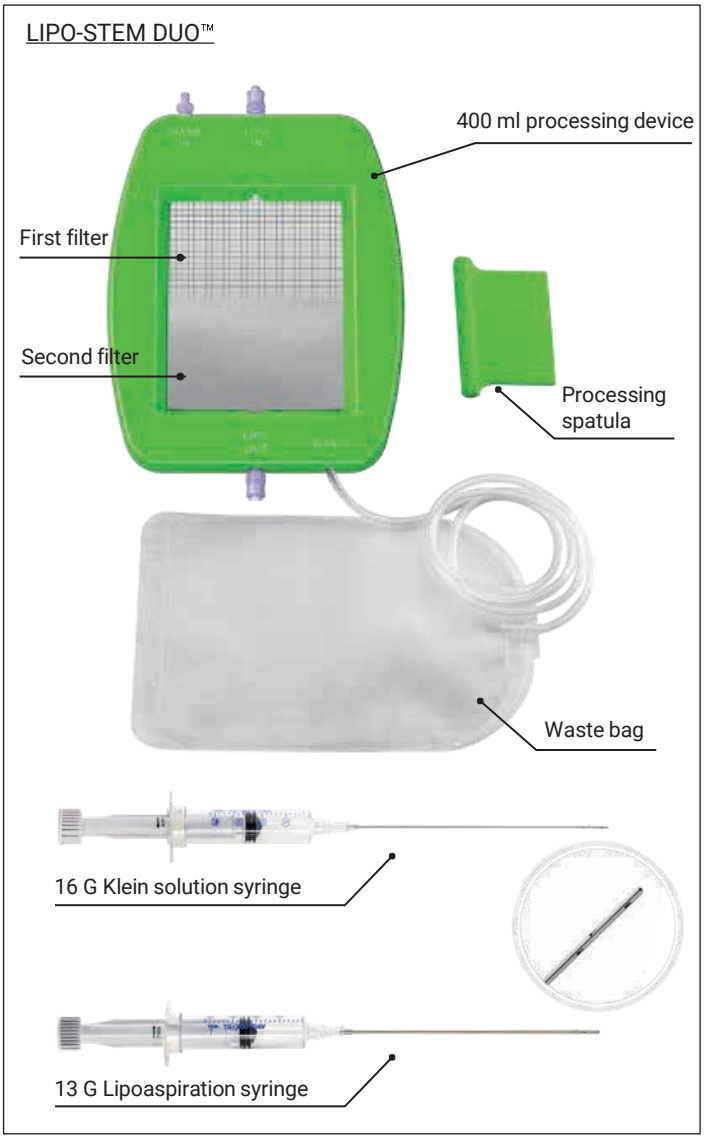
Introduction

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

The filtering system consistently microfragments the adipose tissue while maintaining 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 for reducing 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 the LIPO-STEM DUO™ device, which features a double filtration process, and a different 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, and 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 analyzed the vascular-stromal niche structure of both samples.

The analysis was 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

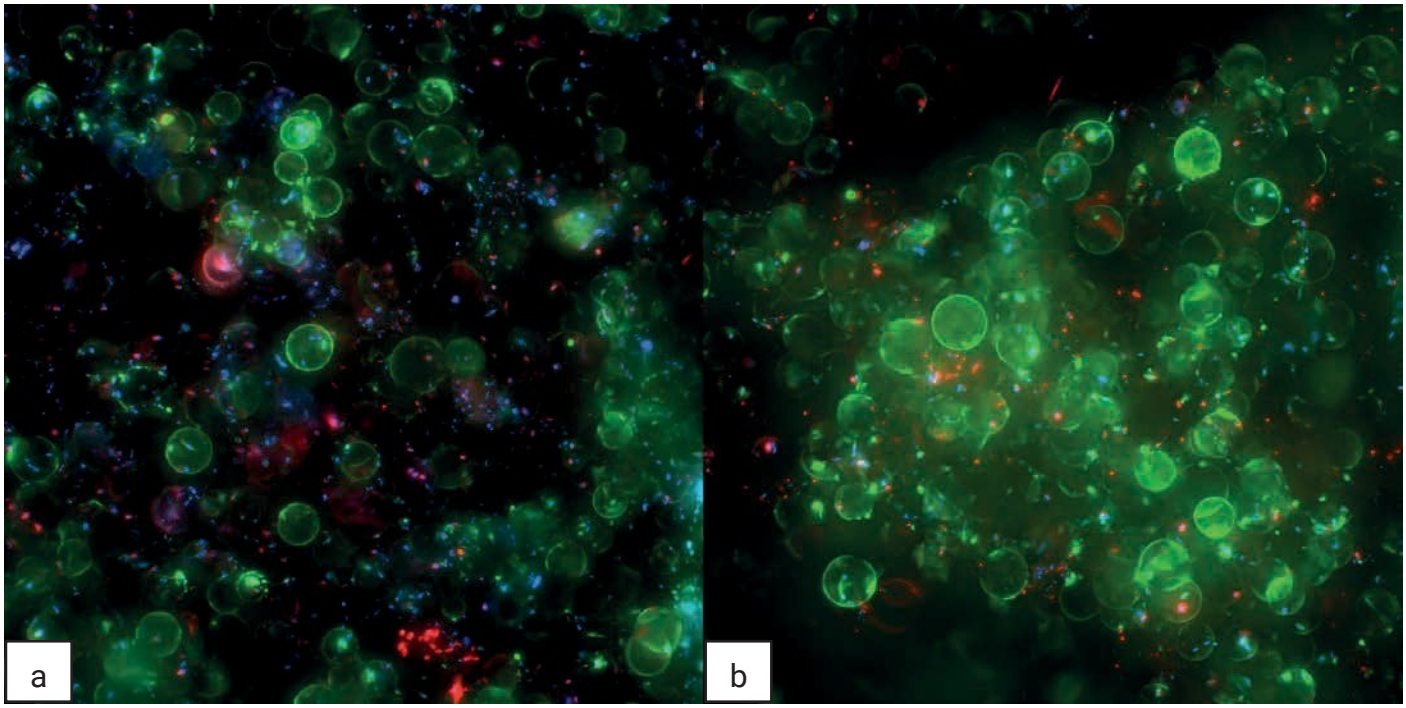


Figure 1 - Tissue Derived from Single Filtration (a), Tissue Derived from Double Filtration (using LIPO-STEM DUO™ device) (b)

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 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.

About 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, the dye may pass through micro holes 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 minimum 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 were obtained using two different processing methods, one with a single filtration device and the other using a 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 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 characterize 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 be seen in two distinct fields of analysis, shown below in Figure 2.

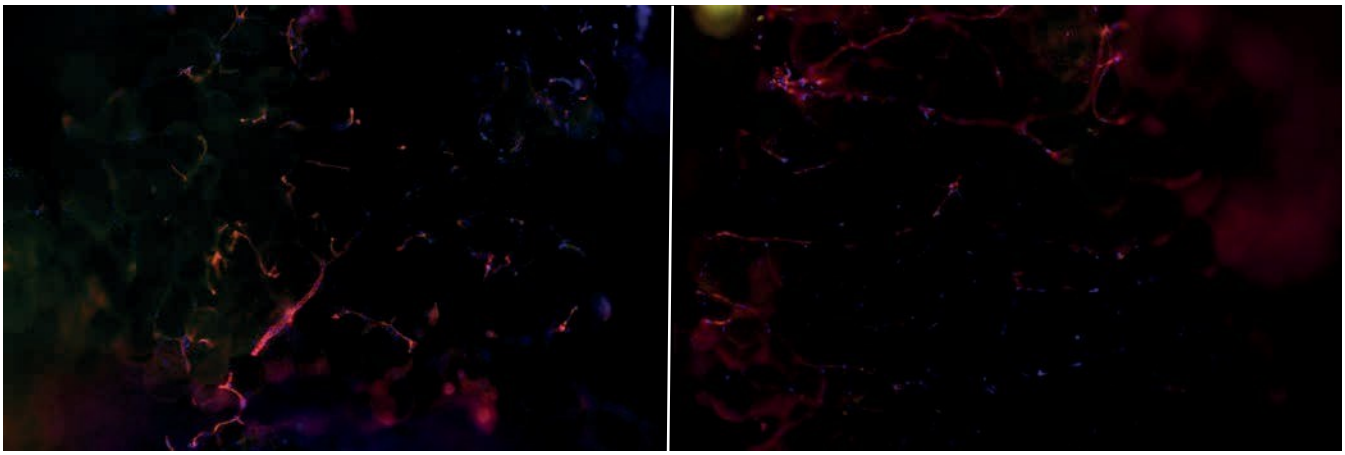


Figure 2a

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.

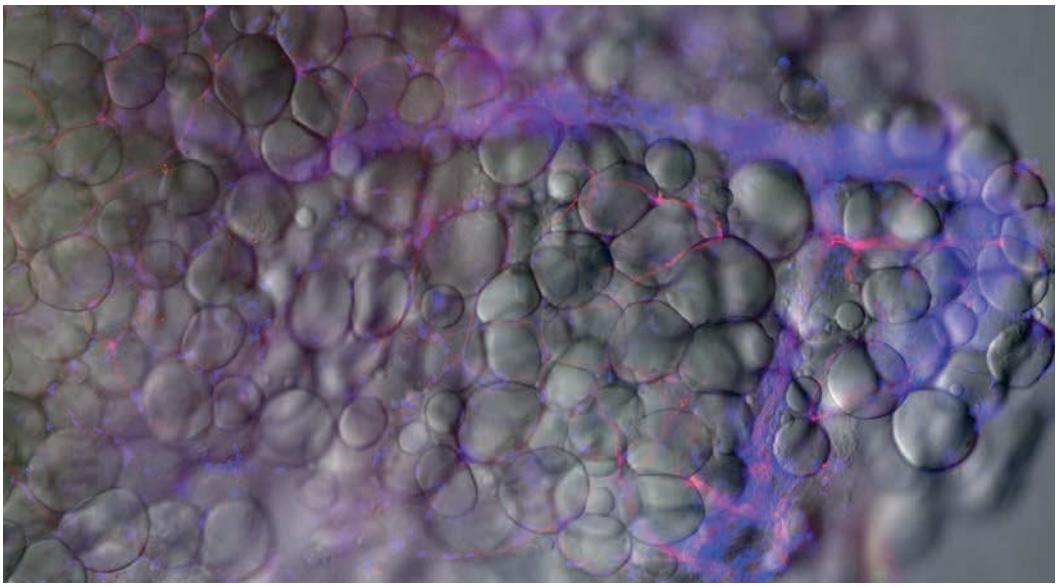


Figure 3

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.

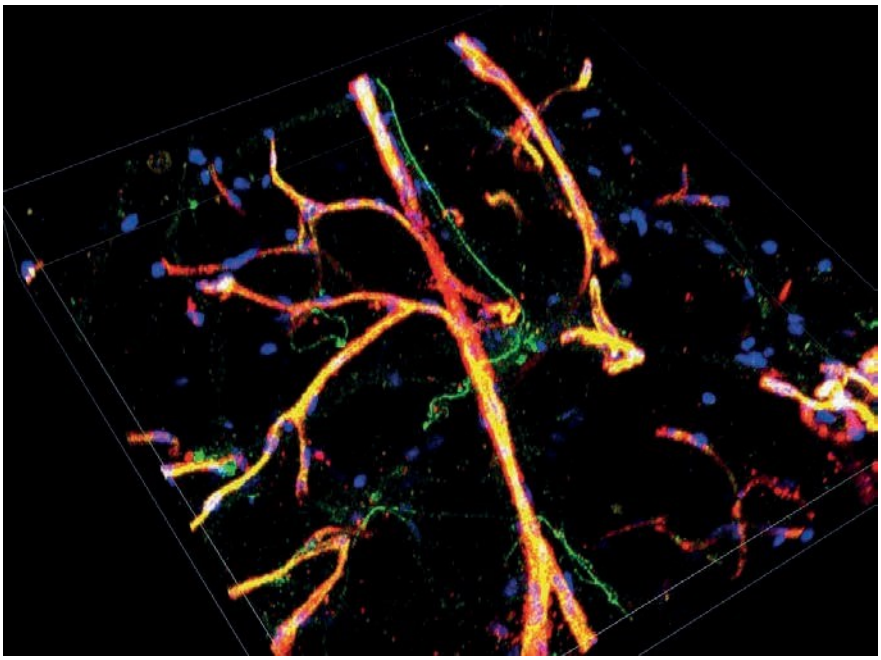


Figure 4

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's 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



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Our company



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

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

50 YEARS

OF EXPERIENCE

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

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

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

2012 — THE EARTHQUAKE: The company was damaged by a heavy earthquake: the family worked hard to keep the production open. After only 2 weeks after the EQ, the company 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 the 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 in 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 conduct 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.

ISO 13485
BUREAU VERITAS Certification

FDA

CE 0123

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 us to reduce production waste and grant high-quality products.

Thanks to the internal R&D Department BPB MEDICA™ conducts constant research on the reference pathologies intending to ever-better qualify and improve its production standards and aid the development of new products.

OUR PRODUCT LINES:

SPINE

ORTHO-BIOLOGICS

ASSISTED REPRODUCTION

BIOPSY

INTENSIVE CARE

AESTHETIC



CLEANROOM
Manual & automatic assembly, packaging.



METAL REFINISHING DEPARTMENT
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MOULDING DEPARTMENT
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