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A New Protocol to Collect Adipose Tissue for Regenerative Purposes

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***Abstract*-**Mesenchymal stem cells are ease of isolation and cultivation for an ex vivo expansion potential in line with the numerous therapeutic mechanisms (paracrine pro-regenerative, anti-fibrotic, anti-apoptotic, pro-angiogenic, and immunomodulatory functions) have contributed to a broad exploitation. But the pre-clinical studies, demonstrated that apparently not the proposed multi-lineage differentiation potential but rather their secreted bioactive molecules that modulate immune and inflammatory responses were key to exerting therapeutic effects,

The interactions between parenchymal and mesenchymal cells, justifies a principally novel approach for regenerative medicine based on co-application of MSC and parenchymal cell for the most efficient tissue repair, than our focus is the all Stromal Vascular Fraction for a more optimized regenerative goal.

Moreover, the potential therapeutic window for the treatment of acute conditions, such as myocardial ischemia and ischemic stroke, require immediate availability of cells and do not allow time for a liposuction procedure on the following hours or days .

For the exposed drawbacks we propose a more efficient method to acquire the intact SVF niche in a fast, efficient and reproducible way, by means of the well-known medical technique of Core Needle Biopsy with an automatic tru-cor biopsy instrument.

Keywords- *Mesenchymal Stem Cells (MSCs), Stromal Vascular Fraction (SVF), Core Needle Biopsy (CNB), Regenerative Medicine*

I. INTRODUCTION

Mesenchymal stem cells (MSCs) have emerged as the most intensely studied cell type for experimental cell therapy. Recent studies suggest that MSCs residing in perivascular compartments of the small and large blood vessels play a regulatory function supporting physiologic and pathologic responses of parenchymal cells, which define the functional representation of an organ or tissue. MSCs secrete or express factors that reach neighboring parenchymal cells via either a paracrine effect or a direct cell-to-cell interaction promoting functional activity, survival and proliferation of the parenchymal cells and therefore can play a role as 'stem/progenitor niche' forming cells[1].

Despite the numerous promising results in preclinical studies, translation into routine practice still lags behind: therapeutic benefits of MSCs are not as satisfactory in clinical trial settings as they appear to be in preclinical models. The bench-to-bedside-and-back approach and careful evaluation of discrepancies between preclinical and clinical results have provided valuable insights into critical components of MSC manufacturing, their mechanisms of action, and how to evaluate and quality-control them [2].

MSC may be harvested from many tissues including from fat tissue in different parts of the body (abdomen, hump, lumbar region, thighs, arms), but it seem that the biologic characteristics of this cells is different according to the anatomical origin, with the superficial abdominal depot (above Scarpas layer) significantly more resistant to apoptosis when compared with upper arm, medial thigh, trochanteric, and both superficial and deep abdominal [3].

Usually the tissue-harvesting procedures have been underestimated as a factor to impact the outcomes. Also the laboratorial protocols for extracting the SVF must be properly evaluated, from simple cells counting up to its characterization as mesenchymal tissue, as well as employing enzymatic tissue digestion through collagenase, limited by the presence of possible xenogeny components, which pose certain risks and safety issues to clinical scenarios [4].

One Study, that compared the biological behaviors of adipose stem cells isolated from fat tissue by lipectomy and liposuction in terms of cell viability, proliferation, migration, paracrine secretion, and anti-oxidative stress capability, indicate that adipose stem cells from lipectomy have better biological characteristics [5]. The viable adipose stem cells yield from liposuction was significantly lower than that from lipectomy, while the apoptosis of cells from liposuction was significantly higher than from lipectomy. Moreover, lidocaine and adrenaline, which are primary components of the tumescent anesthesia fluid used in liposuction, decreased the viability of adipose stem cells, and its cytotoxicity was dose-dependent, while the stem cells proliferation rate treated with lidocaine or adrenaline was greatly decreased [5].

In a study that compares the structure and cellular components of aspirated and excised adipose tissue revealed the presence of a capillary network running alongside adipocytes, which was partly disrupted in lipo aspirated adipose tissues. Aspirated adipose tissue also lacked large vasculature structures and showed significantly larger numbers of small lipid droplets (ruptured adipocytes) and dead cells, compared with excised adipose tissue [6]. On the other hand adipose-derived stromal cell yield in aspirated adipose tissue was approximately one-half that in excised tissue. The authors' results indicate the differential structure and cellular composition of the two tissues, and significant tissue damage and progenitor yield deficiency in aspirated adipose tissue [7]. Adipose Tissue excision method to collect EVF has a better potential material and characteristics for regeneration indications than the most actually used, the liposuction method.

II. Aims

Although till now, the lipoaspirate technique is the gold standard method to collect all the potential cells from adipose tissue for tissue regeneration, from the adipose compartment to the others organs, seems that we may know look for a more optimized method for this purpose.

Needle biopsy with automatic systems, has replaced excisional biopsy as a definitive diagnostic technique for breast tumors. These automatic systems were cold Core Needle Biopsy (CNB) or Tru-cut Biopsy, The most using is a 14-G needle, to obtain larger samples of tissue [8].

It is a safe, well tolerated by patients and minimally invasive method that does not require hospital stay. It causes minimal to no scarring and does not deform the anatomy. It allows removal of multiple tissue samples in a single attempt in a relatively short time and the patient is able to quickly return to work [9].

We propose a new protocol to collect fat for regenerative medical use by using a 14G automatic Core Needle Biopsy (CNB), instead of the classical lipoaspiration method.

III. Results (Clinical protocol)

A. Preparation of the Patients

1- Patient should refrain from active and passive smoking 24 h prior the biopsy.

2- Perform a Sonography evaluation of the fat pad of the per-umbilical abdominal area, to evaluate the depth and vascular pattern of the adipose tissue, the presence of hernias or any scars. Ultrasonographic examination will be performed using a 10 MHz linear probe, before each biopsy blood vessels around the area are marked into the skin, to be avoided during the procedure, and the depth of the fat pad is checked and registered (Figure 1: Measure of the 2 fat pads above and under the Scarpas Fascia).

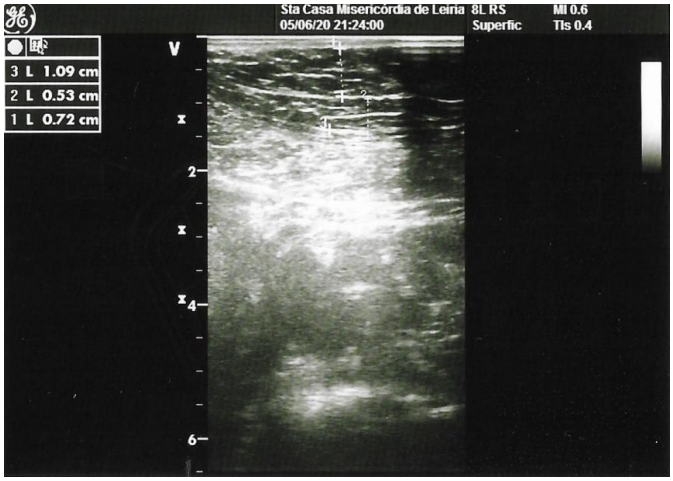

Figure 1. Measure of the 2 fat pads above and under the Scarpas Fascia

B. Preparation of the Surgery Equipment and Consumables (Figure 2)

Position a disinfected surgical field on a 4-wheel surgical Mayo stand:

- One scalpel (No. 11) One scalpel handle
- Povidone-iodine (on a sterile gauze)
- One adhesive sterile gauze
- One 2-mL syringe with a 19G needle and 2 mL of 2% lidocaine plus 1% adrenaline (dermal anesthesia).
- One pair of disinfected surgical gloves
- One dermal pencil and a ruler
- One 14G Tru-cut Automatic Biopsy Instrument 10 cm length (Tru-core ™ Automatic Biopsy System, from Argon Medical Devices.

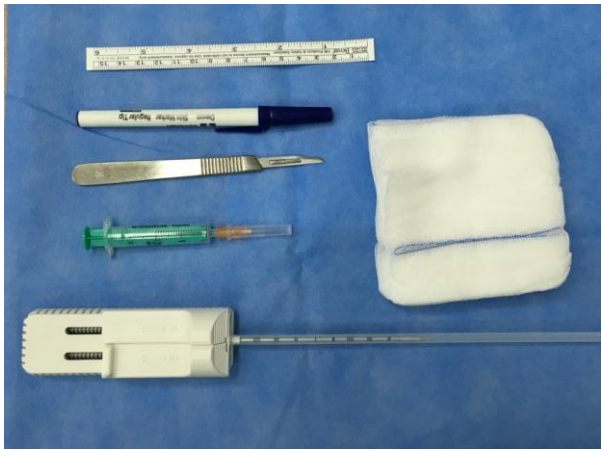

Figure 2. Surgery Equipment and Consumables

C. Preparation of the surgical field (figure 3)

1- Position the participant on the surgical bed in a supine position.

2- Disinfect the region of the incision using povidone-iodine

3- The skin will be covered with special surgical drapes

4- Mark the biopsy area projected into the skin with the dermal pencil and the ruler.

5- Inject the dermal anesthesia, slowly and steadily into the selected dermal region to serve as local anesthesia.

6- Create 1 incision of 2- 3mm in length in the skin of the umbilicus with a plain scalpel to access the fat.

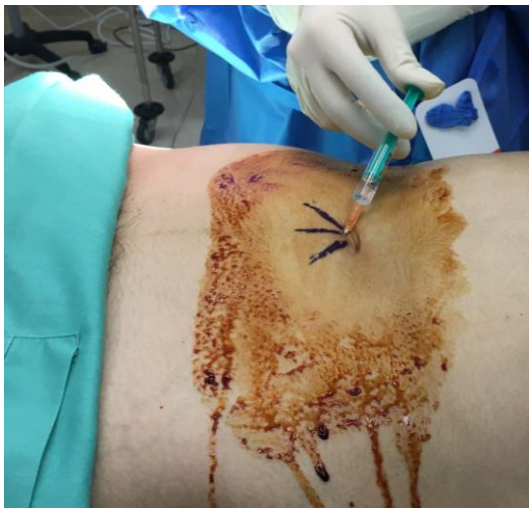

Figure 3. Preparation of the Surgical field

D. Trucut biopsy technique (figure 4)

The surgeon held the tissue with the thumb and the index finger of the non dominator hand and introduce the biopsy gun 2 cm from the incision to the left or right lower quadrant away from the umbilicus and made 20 subsequent biopsies from that side but in different directions, clock wise. The adipose tissue will be than collected, some digital pressure is applied to the biopsy area for 5 minutes, and then the incision is disinfected and taped. No sutures will be used. Local ice was recommended for the next 3-4 hours. Pressure dressing will be used routinely over the biopsy area for 24 h following the procedure.

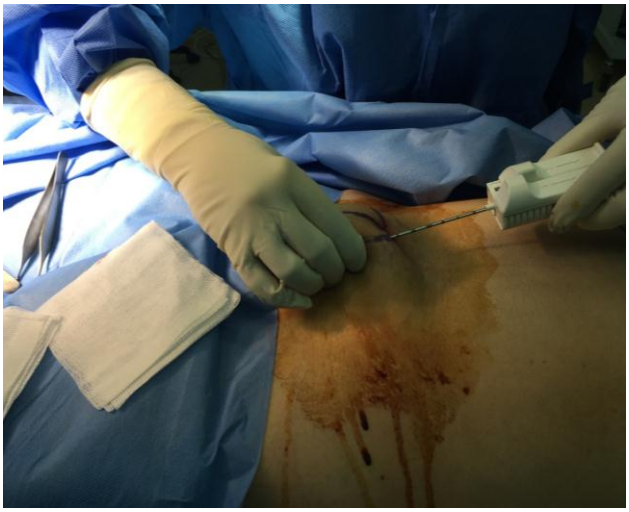

Figure 4. Trucut biopsy technique Discussion and Future Perspectives

The SVF direct use on cellular therapies seems to be, in the short term, the smartest strategy to widespread translational research in the regenerative medicine field. It's regenerative potential has been demonstrated in numerous diseases and applications with similar therapeutic effects of ASCs. In addition, the preservation of the extracted SVF microenvironment could eventually favor its biological activity at the transplanted site, referring to the niche hypothesis concepts [4], and its clinical applications in various medical and surgical specialties, justifying the present and future significant efforts on new techniques for isolating, collecting and maximizing these stem cells.

We intend to contribute with our work to a new secure and reproducible technique to this goal.

The protocol includes the collection of superficial abdominal fat by a tru-cut automatic biopsy instrument. The advantages would be (1) increase cell viability, as described above (2), lower the patient trauma, (3) fasten the procedure, 45 to 60 min turns in 10 to 15 minutes, (4) more rentable method, no need for wash tissue because no liquid infusion needed, (5) widens the eligibility of patients, some patient are not eligible for lipoaspiracion but for soft tissue biopsy any one may be suitable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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