

## SEFFICARE® RATIONALE

### DESIGNED FOR THE REGENERATIVE THERAPY

Clinical studies demonstrated the efficacy of therapies based on the autologous grafting of adult mesenchymal stem cells to accelerate the healing and regenerative processes of the skin and mesenchymal tissues. Moreover, such therapies can be applied in the treatment of autoimmune pathologies for their immune modulating capabilities.

Adipose-derived stem cells (ADSCs) are pluripotent adult progenitor cells derived from embryonic connective tissue. ADSCs can differentiate towards adipogenic, osteogenic, chondrogenic, myogenic cells but also towards non mesodermic cell lines (neuronal, endothelial, epithelial cells, etc.). They are also able to secrete a series of growth factors, such as VEGF (Vascular-Endothelial-Growth-Factor), HGF (Hepatocyte-Growth-Factor), FGF-2 (Fibroblast-Growth-Factor-2) and IGF-1 (Insulin-Like-Growth-Factor-1), which grant them a regenerative and angiogenic power.

One of the tissues richest in adult mesenchymal stem cells is the adipose tissue. Today regenerative medicine mostly resorts to adipose-derived stem cells (ADSCs) because of their characteristics and easy availability. ADSCs carry out their regenerative and immune modulatory capabilities thanks to the paracrine effects, through trophic factors that show anti fibrotic, anti-apoptotic and pro-angiogenic activities. Moreover they perform their regenerative activity thanks to their intrinsic capability of transforming into cells of the mesenchymal and endothelial line, promoting tissue reparation.

ADSCs can modulate a vast array of immune system cells, among which B and T lymphocytes, neutrophils and “natural killer cells”.

This immune modulating and anti-inflammatory capability is being investigated also in the field of allotransplants. Studies have demonstrated an allotransplant engraftment favoring action when associating ADSCs.

Several studies have identified adipose tissue as one of the richest in ADSCs, in particular in its stromal component (SVF Stromal Vascular Fraction). Studies have demonstrated a higher concentration of ADSCs in adipose tissue than in bone marrow (MSCs Marrow Stem Cells). By comparing MSCs and ADSCs, the latter had a higher concentration in the same amount of harvested tissue, they entail a less traumatic harvesting method and their concentration seems to be less affected by patient’s age.

ADSCs proven regenerative capabilities are used in different medical areas such as reconstructive and esthetic surgery, maxillo-facial surgery, vulnology, orthopedics, vascular surgery, orthopedics, cardiology, gynecology, urology, otorhinolaryngology, proctology and dermatology.

A number of experimental and clinical studies have demonstrated the effectiveness of ADSCs-based cytotherapy in favoring wound healing processes. It has been actually demonstrated how one of the main factors involved in retarding healing is microcirculation deficit, therefore the ADSCs angiogenetic action and their capability of differentiating into mesenchymal tissues plays a fundamental role in accelerating and improving wound healing. Moreover, in favoring the process of wound healing, an important role is played by both the angiogenic action – taking place through the expression of genes VEGF, FGF2, PDGFRA, and PDGFRB31 – and the migration of fibroblasts and keratinocytes – favored by the expression of gene EGF (Epidermal Growth Factor). Further studies have demonstrated the efficacy of ADSCs therapy in the wound treatment of diabetic and arteriopathic subjects. In such patients the slow healing processes can be mainly attributed to a

circulatory deficit, therefore ADSCs administration, thanks to their angiogenetic action, favors vascularization reducing healing time.

A recent study was conducted on patients with peripheral arteriopathies due to arteriosclerosis or diabetes (CLI). These patients were treated with ADSCs injections and all them reported improvement in terms of pain reduction and recovery times. Other recent pilot studies have been performed on patients with critical limb ischemia (CLI) treated with local SVF injections and improvement has been reported in 86.7% of patients.

In 2014 a trial study in phase 1 showed that the administration of ADSCs in 60 patients with non-revascularizable CLI (Critical Limb Ischemia) (including 3 diabetics) resulted in pain reduction, reduction of ulcer size and resolution of walking pain with control at 2 years.

Particular attention is paid to the fact that over two million Italians suffer from ulcers and difficult wounds, with the largest (and constantly increasing) percentage represented by decubitus ulcers, given the aging of the population. For the NHS the estimated expenditure is about 1 billion a year, divided as follows: over 50% for hospitalizations, 15-20% for the purchase of dressing materials, 30-35% for nursing staff time. Ulcers and difficult wounds represent a subject of multidisciplinary interest, involving different specialties, from surgeons to internists and orthopedists, from geriatricians to diabetologists.

Diabetes is a chronic disease that affects 350 million people worldwide. 15% of these patients suffer from foot ulcers that often turn into chronic ulcers (Diabetes Foot Ulcer DFU). The annual incidence of amputations varies from 0.21 to 1.37% and in diabetic patients the risk of amputation is 15 to 70% higher than in non-diabetic patients.

The difficulty in healing wounds in diabetic patients depends on several factors:

- chronic inflammatory processes,
- reduction in Growth Factor secretion,
- reduction in collagen synthesis,
- reduction in neoangiogenesis.

Recently numerous studies have highlighted the effectiveness of ADSCs therapy in promoting wound healing in diabetic patients. This healing action is carried out thanks to the ability of ADSCs to differentiate into neuronal cells, smooth and endothelial muscle cells and the ability to secrete numerous growth factors that favor the formation and migration of endothelial cells, fibroblasts and keratinocytes.

It has been shown that ADSCs have the ability to secrete numerous Growth Factors such as hepatocytes GF (HGF), vascular endothelium GF (VEGF), transforming GF-Beta (TGF- $\beta$ ), insulin like GF (IGF-1), fibroblasts GF (bFGF), granulocytes and macrophages stimulating factor (GM-CSF), tumor necrosis factors (TNF) –  $\alpha$ , interleukins 6-7-8-11, adiponectin, angiotensin, cathepsin D, retinol binding protein and CXCL-12.

A preclinical study demonstrated the efficacy of topical therapy with ADSCs in combination with collagen sponge in diabetic animals with ulcers.

In six randomized controlled trials (RCTs) it was shown that the administration of ADSCs promoted healing of foot ulcers in diabetic patients (DFU); these benefits take place in ulcers even larger than 5 sq.cm and also in patients over 70 years of age.

Another important application of regenerative therapy with ADSCs is in the orthopedic field: numerous studies have shown the effectiveness of mesenchymal ADSCs therapy thanks to their intrinsic ability to regenerate cartilages, tendons and bones. Thanks to this potential, the SVF

(Stromal Vascular Fraction) containing ADSCs is used in patients suffering from osteoarthritis, chondromalacia, meniscal lesions, osteonecrosis of the femoral head and tendon injuries.

Injuries of the skeletal muscle system are more and more frequent in men and women of all ages and often the proposed therapies are mainly symptomatic and aimed at relieving pain. Regenerative therapy opens up new therapeutic possibilities in the treatment of such lesions. In particular, numerous studies have shown that mesenchymal ADSCs have the intrinsic ability of differentiating into chondrocytes and osteocytes.

Another important ability of wound cytotherapy with ADSCs is the antifibrotic ability to be attributed to the production of TGFbeta3 and HGF, which are important antifibrotic mediators. The problem of hypertrophic scars is obviously not merely an aesthetic problem but often determines functional limitations depending on the anatomical site. Therapy with ADSCs has demonstrated its efficacy on both experimental and clinical models.

### **S.E.F.F.I. AND MicroS.E.F.F.I. TISSUE GRAFT - Superficial Enhanced Fluid Fat Injection**

Since 2015 A. Gennai et al. have been publishing several studies (see references) about the new tissue graft techniques SEFFI and MicroSEFFI. These techniques aim to graft adipose tissue including the stromal fraction (SVF) and the ADSCs Adipose Derived Stem Cells contained therein, in order to achieve a tissue regeneration. The Authors proved that it is possible to harvest tissue using special cannulas with very small side ports holes so as to select small cellular clusters that do not need any kind of manipulation in order to fluidify the tissue.

SEFFI and MicroSEFFI are now among the most used techniques intended for regenerating tissues. These techniques are considered minimally invasive surgical procedures; hence they require liposuction skills and surgical facilities.

In the light of this evidence, Regenerative Therapy has always been only in the hands of plastic surgeons and not open to other doctors and surgeons without liposuction skills.

Dr. Gennai strongly believes that the Autologous Regenerative Therapy should be performed also by Doctors and Surgeons even without any liposuction skills: for this reason, he developed, standardized and patented\* a special guide addressed to harvest the tissue in a safe, easy, effective way without any liposuction skill.

From this original idea SEFFILINE developed SEFFICARE®:

it's an all-in-one and disposable medical device to allow doctors to perform the autologous regenerative treatment in their facility in a safe, easy, effective way.

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\*Italian patent

### **WHY SEFFICARE®**

In order to perform a correct Regenerative Therapy, a **SAFE, EASY, EFFECTIVE** and **STANDARDIZED** method is needed to harvest adipose tissue containing the stromal component (SVF) and adult mesenchymal stem cells (ADSCs). This method must have the following characteristics:

- Tissue harvesting should involve the least possible trauma both for the tissue and the patient; adipose tissue harvesting should occur as close as possible to the skin surface in order to obtain a tissue richer in ADSCs;
- Cell clusters should be harvested with small size adipocytes, stromal tissue (SVF) and ADSCs to favor their engraftment in the recipient site;

- The harvested tissue should be manipulated as little as possible in order to preserve the highest cell viability and avoid the use of devices for tissue fragmentation: this will make the method faster and less expensive;
- The method should be as little traumatic as possible for the patient and minimize harvesting complications such as hematomas, seromas, infections and skin irregularities;
- The tissue to be engrafted should be sufficiently fluid to be grafted with needles or thin cannulas so as to be as least traumatic as possible for the recipient site. In the technique of preparing the tissue to be engrafted, the harvesting phase is the most difficult one for doctors who do not have a specific preparation in suctioning subcutaneous adipose tissue. Due to this difficulty, very often physicians resort to the aid of specialists in plastic surgery, thus increasing the costs of the procedure and making the organization of the therapy more complex, since it might require several treatments. In some cases, they renounce to regenerative therapy, exposing their patients to possible complications related to incorrect harvesting maneuvers.

**SEFFICARE®** is a sterile disposable “all in one” kit, for regenerative medicine therapy. The kit makes the harvesting, preparation and grafting procedure **EASY, SAFE, EFFECTIVE** and **STANDARDIZED**.

### **EASY**

Our patented guide allows the doctor, even without any specific experience in the subcutaneous tissue aspiration, to harvest the tissue from the correct plane and to minimize the risk of performing maneuvers that may damage the patient. The all-in-one disposable medical device needs no external equipment such as pumps. Moreover, the procedure can be performed under local anesthesia in a medical facility. It requires only a minimal tissue manipulation. All this simplifies and reduces the time of the procedure.

### **SAFE**

The disposable medical device reduces the risk of contamination and transmission of diseases. The patented guide makes the harvesting phase safe even for doctors without a specific experience in the harvesting of subcutaneous adipose tissue.

### **EFFECTIVE**

The patented device “guides” the harvesting in the correct superficial plane where the maximum concentration of mesenchymal stem cells (ADSCs) occur. In addition, the harvesting microcannula selects small-sized cell clusters, thus reducing subsequent manipulation and therefore preserving maximum cell viability. Cell cluster small size favors engraftment and allows the grafting with needles or small sized cannulas in the superficial plane favoring the regenerative effect and decreasing the traumatism of the receiving site. The small size of the cell clusters favor engrafting and allow to perform grafting with small-sized needles or cannulas in the superficial plane, thus favoring the regenerative effect and decreasing injury in the recipient site.

### **SEFFICARE® AS COMPARED WITH COMPETITORS**

1. Its special SEFFI cannula guarantees the selection of cell clusters – small in size but rich in SVF and ADSCs – which do not require subsequent manipulations.
2. The method can be performed in any medical facility under local anesthesia, as it does not require an operating room or external aspiration systems.

3. The patented guide makes the method easy, safe and effective, allowing any doctors to easily and safely harvest subcutaneous adipose tissue. Furthermore, the guide guarantees that tissue harvesting takes place in the superficial adipose planes where the maximum concentration of SVF and therefore ADSCs occurs.
4. The kit is disposable and contains all the necessary equipment to perform harvesting, preparation and grafting (only skin disinfectant, local anaesthetic, physiological solution, adrenaline and sterile gloves are to be added).
5. Economical: to date it is the most economical medical device on the market for such use.

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