Hy-tissue SVF: new kit for the withdrawal of the Stromal Vascular Fraction (SVF) from adipose tissue

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The use of multilinear stem cells in clinical applications that aim at tissue regeneration and repair has remarkable therapeutic potential. One source of this type of cells is the medullary stroma. This contains several cell populations, including mesenchymal stem cells (MSCs), that are capable of differentiating into adipogenic, osteogenic, chondrogenic and myogenic cells **(Figure 1)**.

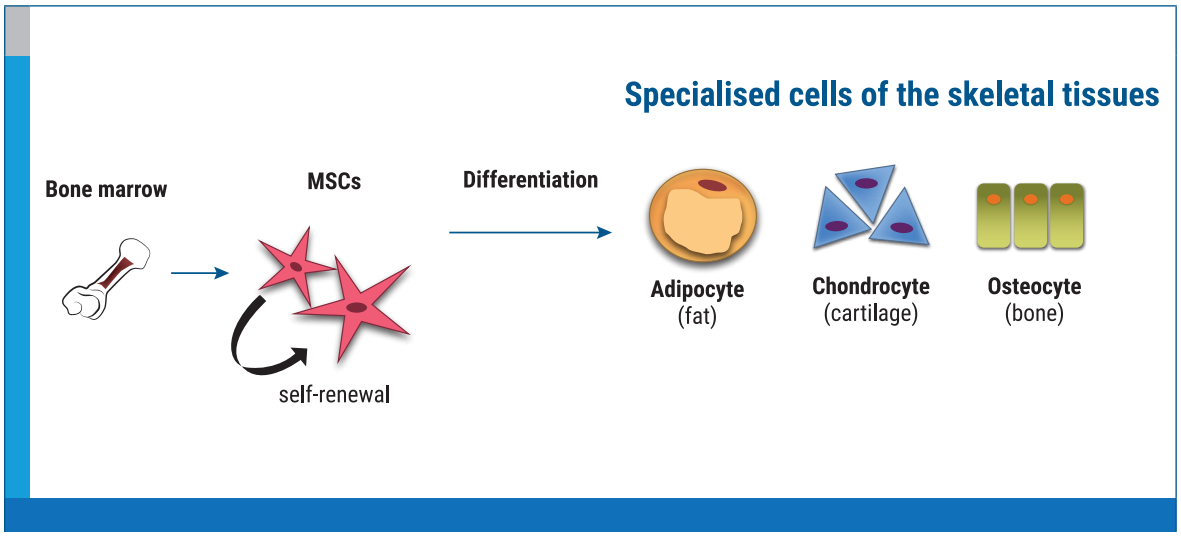

Figure 1. Schematic representation of the differentiation of MSCs from bone marrow. (http://www.regenervate.com)

However, autologous bone marrow procurement has potential limitations. An alternative source of autologous adult stem cells that is obtainable in large quantities, under local anesthesia and with minimal discomfort, is human adipose tissue. Adipose-derived stem/stromal cells (ADSC) have been extensively studied as an important cell type of cells with regenerative potential. The Stromal Vascular Fraction (SVF) refers to a heterogenic population of mononucleate cells contained in adipose tissue. SVF is appreciated for the regenerative potential of cells and other factors it contains. Unlike ADSCs, SVF is easier to obtain and requires neither cell separation nor culture conditions. SVF cell-based product can be obtained instantly from adipose tissue, without subtantial manipulations. In fact, MSCs are up to 500 times more prevalent in adipose tissue than in bone marrow. Hy-tissue SVF is a new kit for the withdrawal of the Stromal Vascular Fraction (SVF) from adipose tissue in a single surgical procedure **(Figure 2)**.

Figure 2. Use of Hy-tissue SVF.

This procedure allows aseptic processing suitable even for small amounts of adipose tissue and only requires filtration and centrifugation (minimal manipulation). It should also be noted that a proper liposuction results in minimal blood loss, making it a minimally invasive and atraumatic procedure. The processing of adipose tissue eliminates potential inflammatory compounds, such as oil and reduces the size of tissue clusters, thereby allowing to obtain a homogeneous and easily manipulated final product, suitable for tissue infiltration. The whole process is shown in **Figure 3**.

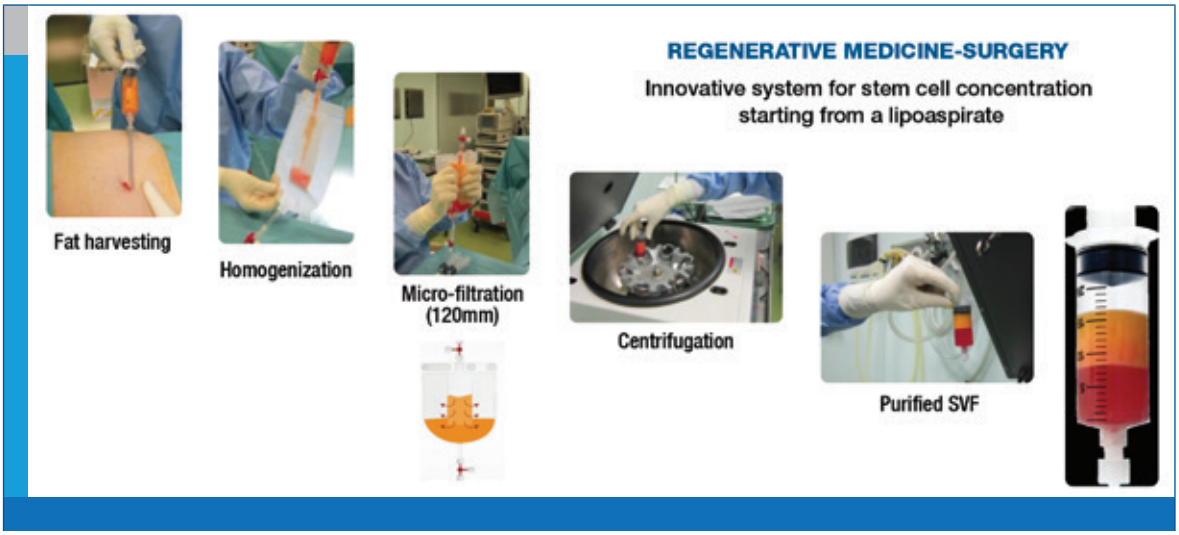

Figure 3. SVF from lipoaspirate obtained with Hy-tissue SVF.

The process is simple, quick and highly reproducible. The entire procedure can be completed in 15-20 minutes. The obtained SVF can be used to suppress immune surveillance in injured tissues, while promoting wound repair, tissue regeneration and angiogenesis. SVF can therefore promote the natural regeneration of focal lesions or degenerated tissue (i.e cartilage). There are many studies with SVF in preclinical and clinical phase that support its potential use in regenerative medicine, specially in orthopedics and cutaneous applications. In conclusion, SVF-based regenerative cell therapy has enormous potential, especially in orthopedic applications.

Hy-tissue SVF: a new perspective in regenerative diabetic foot therapy

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Diabetic foot is a major global health problem. 19-34% of diabetic patients develop a foot ulcer at least once in their lifetime. In Italy alone, there are about 300,000 diabetic foot patients. These individuals are also at high risk of amputation and death. The standard treatment of diabetic foot ulcers includes debriding of necrotic tissues, surgical or endovascular revascularization, infection control, wound discharge, metabolic control, and foot care education. However, these treatments are often insufficient to ensure satisfactory wound healing, resulting in increased risk of limb amputation. The use of adipose tissue-derived mesenchymal cells (ADSCs) is a new and remarkably promising therapeutic strategy. These cells are able to differentiate into different cell lines and are considered an alternative to mesenchymal cells derived from bone marrow. The overall goal of cell therapy is to develop cells and tissues capable of repairing, replacing or improving biological functions **(Figure 1)**. The use of ADSCs is based on a wide variety of mechanisms and processes **(Figure 2)**.

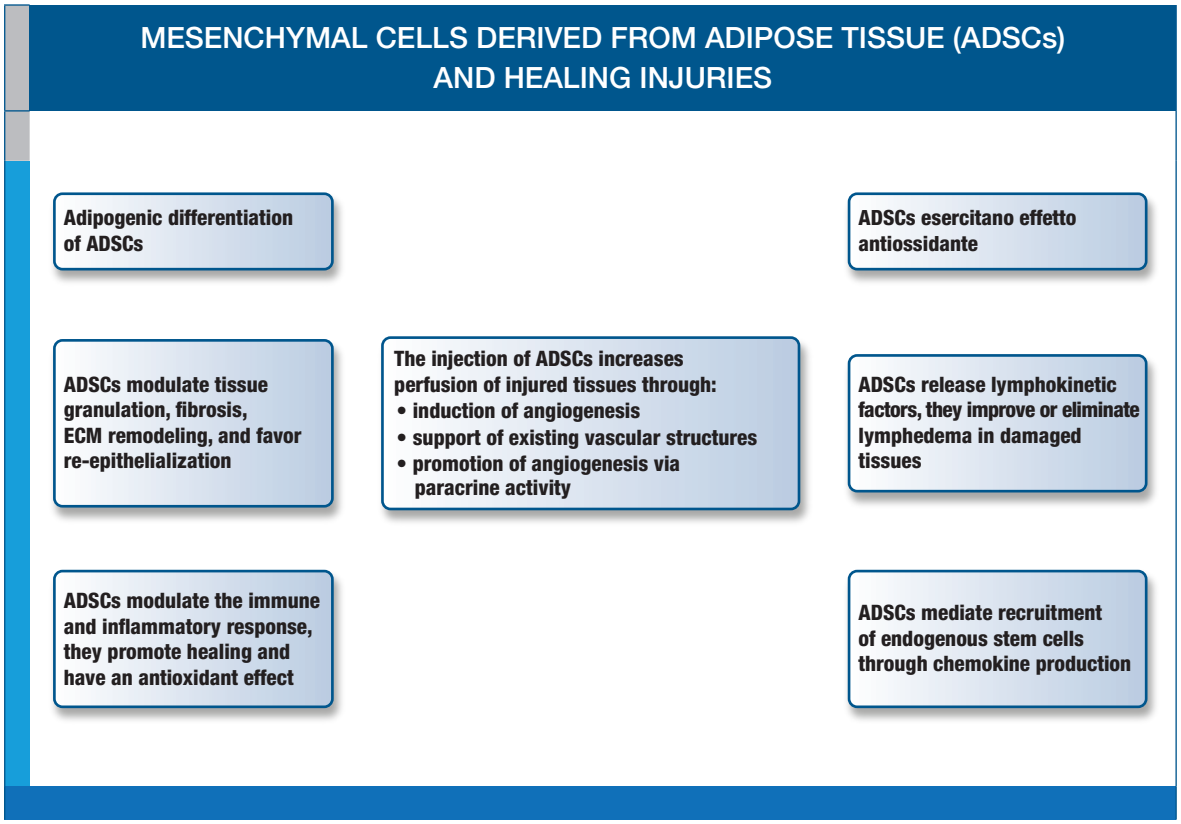

Figure 1. Mechanism of action of the ADSCs.

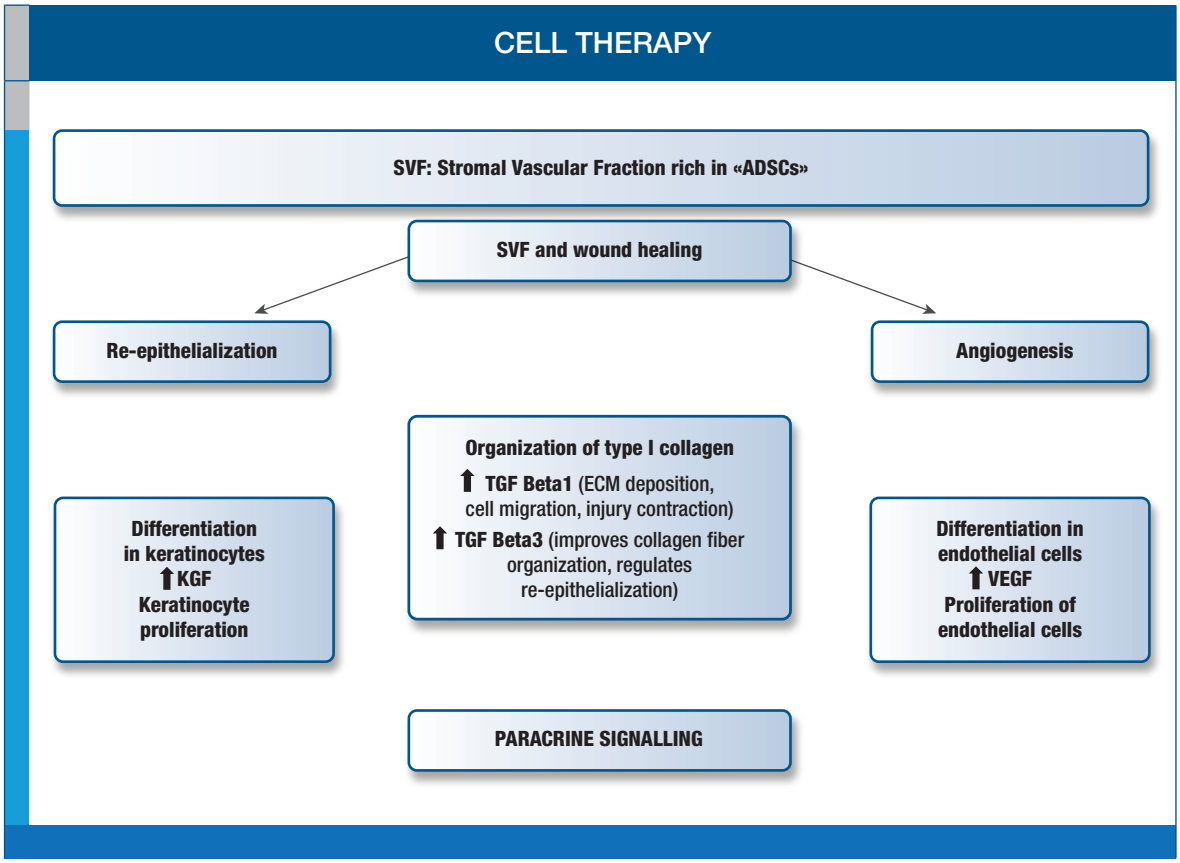

Figure 2. Processes involved in cell therapy with ADSCs.

ADSCs have been evaluated for use in the healing of diabetic wounds, suggesting their therapeutic benefit. Briefly, there are various mechanisms involved to explain the clinical benefits observed; among these, increased epithelialization, formation of granulation tissue, neo-angiogenesis, in situ release of growth factors, and ADSC capacity to differentiate into mesenchymal cells of different tissue lines. The SVF obtained by mechanic procedures is considered safer because it avoids the need for *in vitro* treatments that may alter the cell biology and functions. In addition, minimal engineering and direct use in one-go surgery comply with current regulations.

We used Hy-tissue SVF in a clinical study involving 8 diabetic patients with lesions of an average size of 56 cm2. After an average follow-up of 124 days, 4 lesions were completely healed; 4 patients are still followed. These results compare favorably with standard treatment protocols. Case studies are shown in Figures 3 and 4.

Figure 3.
Healing of diabetic foot ulcer treated with SVF, using Hy-tissue SVF.

Figure 4.
Healing of diabetic foot ulcer treated with SVF, using Hy-tissue SVF.

Now, more than ever it appears necessary to proceed with further developments and studies on withdrawal techniques, as well as on the isolation and preservation of this emerging therapy. Clinical trials are also needed on a large scale, that confirm the effectiveness of ADSCs present in the Stromal Vascular Fraction (SVF), in diabetic foot injuries involving long-term surveillance of potential complications. Finally, it is a promising technique that requires guidelines and standard protocols to further develop this cell-based therapy.

Hy-tissue SVF: the first clinical experiences in diabetic foot

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Diabetic foot is defined as a condition of infection, ulceration and / or destruction of deep soft tissues, associated with neurological abnormalities and peripheral vasculopathy of variable degree involving the lower limbs. The goal of diabetic foot treatment is to preserve the anatomical and functional integrity of the foot **(Figure 1)**. When this is not possible, the objective is to reduce disability caused by the disease and resulting from therapeutic approaches.

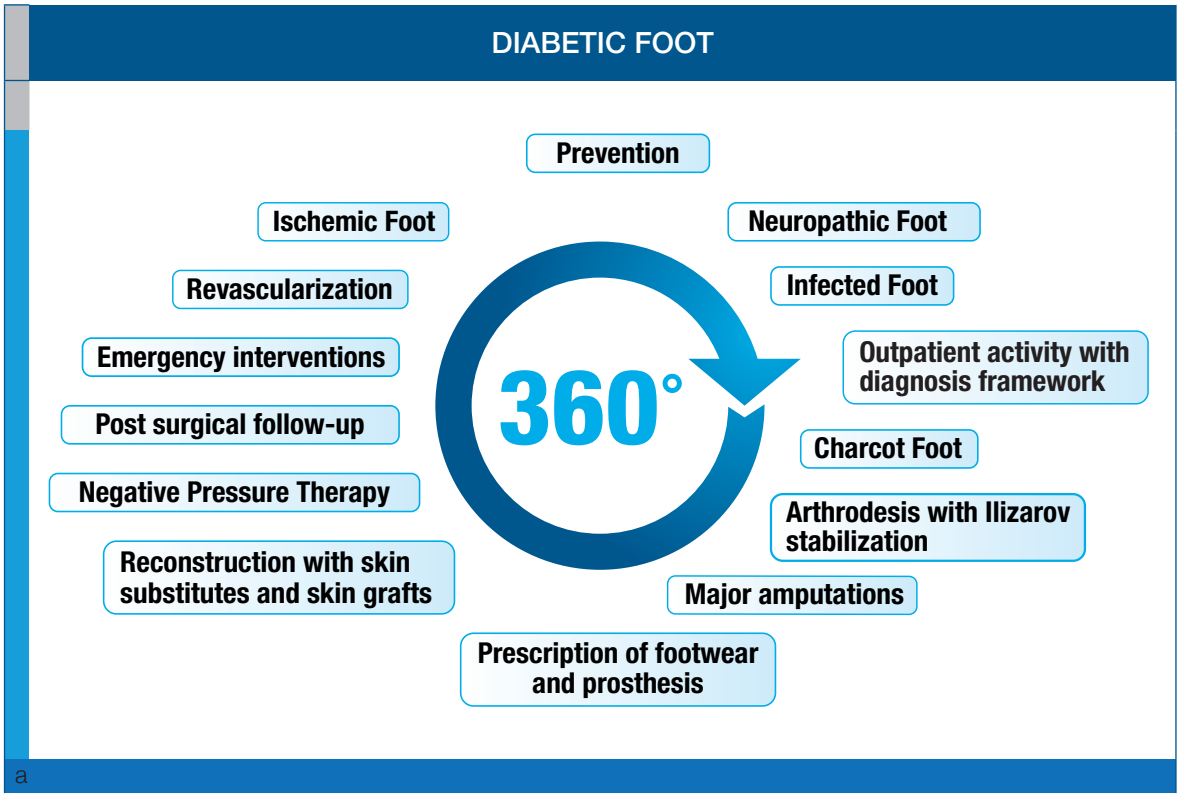

Figure 1. Treatment of diabetic foot.

Of particular interest is the use of accelerated wound closure or re-epithelialization, that evokes the "closed and clean" approach, which can be followed by revascularization **(Figure 2)**.

Figure 2. Revascularization of the diabetic foot.

There is a growing interest in the application of the Stromal Vascular Fraction (SVF) isolated from adipose tissue in the treatment of diabetic foot. We used SVF obtained using Hy-tissue SVF kit, to treat several cases of severe diabetic foot, with encouraging results. In particular, SVF was re-injected in the peri- and intralesional area and loaded on a scaffold containing hyaluronic acid and type I horse collagen **(Figure 3)**.

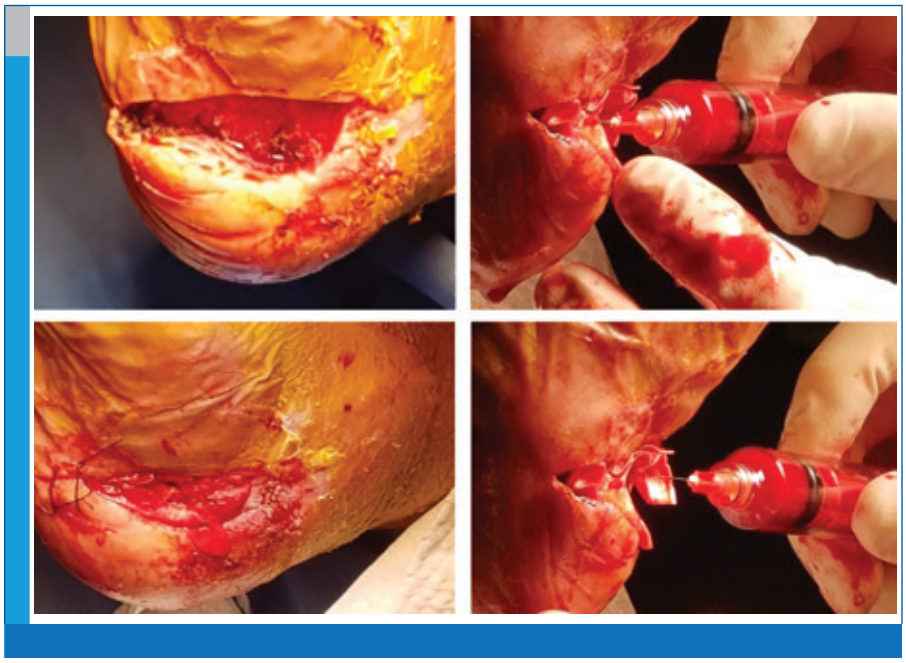

Figure 3.
Application of SVF in a diabetic foot injury.

Case studies of patients treated with SVF obtained using Hy-tissue SVF are shown in **Figures 4** and **5**.

Figure 4. Diabetic foot treated with SVF obtained using Hy-tissue SVF.

Figure 5. Diabetic foot treated with SVF obtained using Hy-tissue SVF.

This treatment showed encouraging results, although further studies are necessary. Finally, the treatment of diabetic foot requires multidisciplinary care. It is necessary to improve surgical techniques and procedures, as well as use innovative devices that enable to avoid or reduce the risk of amputation and favor re-epithelialization. The use of SVF obtained with Hy-tissue SVF in the treatment of diabetic foot allowed a fast and efficient re-epithelialization, and can be considered useful in providing further support in more complex cases.

The use of SVF in the treatment of neuropathic foot

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The use of SVF for the treatment of diabetic foot has been the focus of various pre-clinical and clinical studies. In fact, data obtained from the mouse model suggest that adipose-derived stem cells (ADSCs) can potentially support wound healing and be suitable for clinical application in the treatment of diabetic foot. This study shows that in wounds, an increase in granulation tissue formation and angiogenesis occurs, as well as reduction of inflammation and protein levels linked to oxidative stress. The results of a recent pilot study showed that the injection of adipose-derived SVF could positively affect microcirculation in the ischemic diabetic foot. An important review on the use of SVF in diabetic foot has established that ADSCs can improve wound healing, by increasing epithelialization and formation of granulation tissue, as well as anti-inflammatory and anti-apoptotic effects and the release of angiogenic cytokines. A few small-sized clinical trials have shown that ADSC treatment in patients with diabetic foot ulcers has led to an improvement in the evolution of the ulcer and in the distance associated with claudication, without reporting any complications. Given the available evidence, ADSCs have great potential in the regenerative therapy of chronic wounds associated with diabetes, although larger studies are needed to confirm the long-term efficacy and safety in these patients.

Surgical treatment of the neuropathic foot with SVF involves several stages, as shown in **Figures 1** and **2**.

1. LOCAL ANESTHESIA		2. WITHDRAWAL PHASE	
The selected donor sites (abdomen and flank) are marked and infiltrated with Klein's solution. This provides adequate anesthesia and facilitates the harvesting of adipose tissue from contiguous planes.		The adipose tissue is removed with a forward-retracting movement from the subcutaneous planes, by means of special blunt-tip cannulas mounted on disposable syringes in which the vacuum effect is created.	

Figure 1. Anesthesia and SVF sampling in the surgical treatment of neuropathic foot.

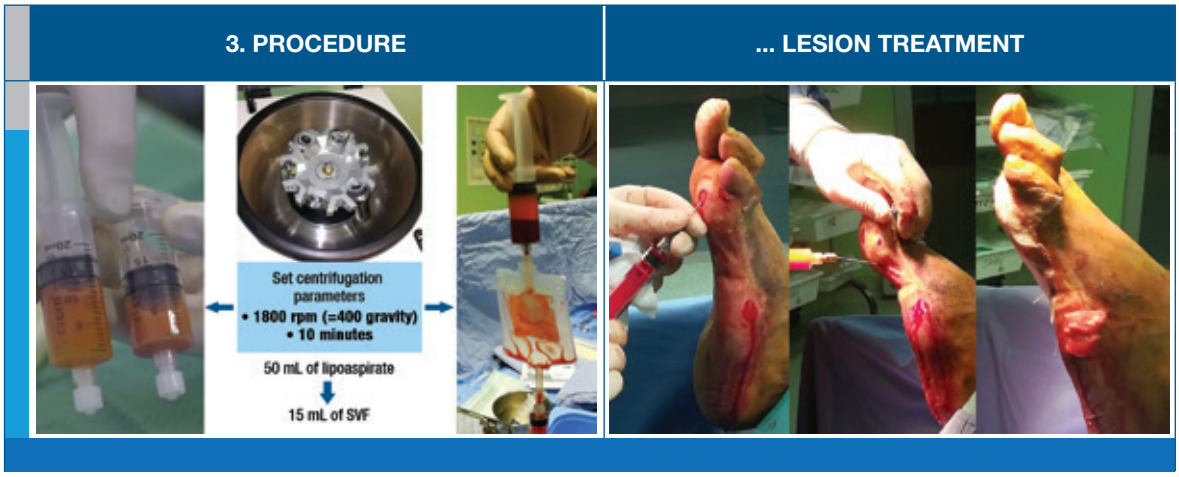

FIGURE 2. Preparation of SVF using Hy-tissue SVF and treatment of neuropathic foot lesions.

FIGURE 3 shows the results of the previous case, at 7 and 21 days.

FIGURE 3. Results of surgical treatment with SVF application, obtained using the Hy-tissue SVF system, at 7 and 21 days.

Excellent results can be observed in as little as 3 weeks. Similar results have been observed in one case of amputation.

In our initial clinical experience, the validity of the results clearly suggests that the use of mesenchymal cells obtained from adipose tissue (SVF) in the treatment of neuropathic ulcers can be considered positive from the point of view of healing time. It is believed that the use of SVF may reduce tissue stress and pressures in neuropathic lesions, thus increasing the number of days without ulcers. In the future, application and absorption timescales will have to be investigated for SVF obtained using the Hy-tissue SVF kit. Randomized trials will be needed for further evaluation of this new therapeutic approach.