Video Article

Stromal Vascular Fraction-enriched Fat Grafting for the Treatment of Symptomatic End-neuromata

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Abstract

The purpose of this study was to methodically illustrate and highlight the crucial steps of stromal vascular fraction (SVF)-enriched fat grafting as a novel treatment of symptomatic end-neuromata of peripheral sensory nerves, and in this study, specifically of the superficial branch of the radial nerve (SBRN). Despite a multitude of existing treatments, persistent postoperative pain and common pain relapse are still very common, independent of the procedure assessed. The neuroma is microsurgically excised accordingly to standardized protocol. Instead of the relocation of the regenerating nerve stump in neighboring anatomical structures, such as muscle or bone, a fat graft is applied perifocally and acts as a mechanical barrier. In order to reduce the fat resorption rate and boost the regenerative potential of the graft, the highly concentrated SVF is integrated in the grafting. The SVF is isolated from subcutaneous fat by enzymatic and mechanic separation of the lipoaspirate by a specific commercial isolation system. The SVF-enriched fat graft provides both a mechanical barrier and various biological effects at the cellular level, including improving angiogenesis, inflammation, and fibrosis. Both mechanical and biologic effects help to reduce the disorganized axonal outgrowth of the nerve stump during nerve regeneration and hence prevent the recurrence of painful end-neuromata.

Video Link

The video component of this article can be found at https://www.jove.com/video/55962/

Introduction

The management of symptomatic end-neuromata of the SBRN remains challenging with a multitude of existing surgical treatments. Principally, the neuroma is excised and the remaining nerve stump is relocated into neighboring structures such as muscles[1,2], bone[3], or veins[4]. This is assumed to prevent the out-sprouting of the regenerating neural fascicles by mimicking the ambience of an end-organ. However, none of these techniques proved superior, as high rates of pain relapse and insufficient pain reduction were often observed.

Recently, fat grafting around the remaining nerve stump yielded promising results and was advocated as a new treatment option for painful end-neuromata of the upper limb[5]. Besides acting as a mechanical barrier to prevent continuing motion and tap pain, the beneficial aspect of fat grafting is rather attributed to the cellular level. Human adipose tissue contains multiple cell types, including preadipocytes, mast cells, pericytes, endothelial cells, vascular smooth muscle cells, fibroblasts, and adipose derived stem cells (ADSCs) along with hematopoietic stem cells[6]. This heterogeneous cell population is referred to as Adipose-Derived Regeneration Cells (ADRCs) or SVF, and can be obtained from the lipoaspirate by an isolation system (see **the Table Of Materials**) and applied enzyme reagent (see **Table of Materials**)[7]. The exact mechanism of action is still not fully understood; however, SVF has been associated with improvements in angiogenesis, immunomodulation, differentiation, and secretion of extracellular matrix[8].

The concentrated cell population of the SVF is added to the fat graft with the purpose to reduce the resorption rate and to increase the regenerative potential of the fat graft, as recent studies revealed pain relapse after 12 months with fat grafting without added SVF[5]. Moreover, the biological action also provides beneficial effects on the nerve itself by reducing disorganized axonal sprouting of the stump[9] and subsequent scar adherences which might result in nerve entrapment.

The first clinical study to describe SVF-enriched fat grafting as a novel surgical option for the treatment of symptomatic end-neuromata of SBRN has been recently published[10]. In general, the technique can be applied to any neuroma occurring after accidental or iatrogenic nerve injury. The aim of the present subsequent study is to methodically illustrate the technical aspects of this procedure.

Protocol

The protocol follows the guidelines of the University Hospital Zurich Human Research Ethics Committee.

1. Diagnoses of Neuromata and Patient Selection

1. Perform preoperative nerve blocks proximal to the neuroma in order to confirm the diagnoses. Assess the blocks in the middle third of the forearm and exclude potential triggers of the overlapping innervation areas of the SBRN, such as the lateral antebrachial cutaneous nerve (LACN), posterior interosseous nerve (PIN), and a dorsal branch of ulnar nerve[10].
2. Perform surgery only in patients with considerable pain reduction (at least 5 points on the Visual Analog Scale (VAS) for pain)[11].

2. Surgical Preparation

1. Forbid the patient any food or liquid intake 6 h prior to the surgery.
2. Place the patient in the middle of the surgical suite on a universal operative table in a beach chair position.
3. Place the affected arm/hand on an arm rest.
4. Ask the anesthetists to perform endotracheal anesthesia (fentanyl 2 µg/kg, rocuronium 0.6 mg/kg, propofol 150 - 250 mg).
5. Disinfect the skin with povidone iodine in the area of liposuction (*e.g.* inner thigh and/or upper/lower abdomen) and the site of neuroma excision.
6. Cover the patient with sterile surgical drapes, leaving exposed the site of liposuction and the surgical site.

3. Tumescent Liposuction

1. Make several small stab (puncture) incisions of approximately 3 - 5 mm diameter with a #11-blade scalpel in the area of liposuction. Incise as far as the subcutaneous layer is reached.
2. Inject the tumescent solution containing a dilute lidocaine and epinephrine (500 mL Ringer's lactate solution, 20 mL of 1% lidocaine, and 1 mg epinephrine) via the 14-gauge infiltrator (infiltrating cannula) into the subcutaneous fat layer (3 mm in depth) in the region of fat harvesting.
3. Wait for at least 20 min allowing the tumescent solution to take effect.
4. Hook the 3 mm-collecting cannula (outer diameter 3 mm, length 26 cm) to a 60-mL Toomey syringe and start harvesting adipose via the stab incisions.
   NOTE: The nondominant hand continually controls placement and course of the cannula.
5. Pull back the plunger of the Toomey syringe to the 60-mL mark and place a mosquito clamp along the withdrawn plunger in order to maintain negative pressure in the syringe and avoid a continuous vacuum creation by the dominant hand.
6. Harvest a minimum of 220 mL of adipose tissue by positioning the Toomey syringes in a vertical position in the provided Toomey syringe stand; this allows the sedimentation of the fat and separation from the fluid.
7. Take special care to obtain a good quality fat graft without substantial amount of blood.
   NOTE: Change the region of liposuction when augmented blood content is noticed.
8. Suture the stab incisions with simple stitches and apply pressure as required (*e.g.* compressive belt).
9. Transfer the lipoaspirate into a single-use sterile disposable set of the isolation system.
10. Keep one Toomey syringe with lipoaspirate in the provided stand in order to obtain the fat fraction for later lipofilling.

4. Adipose Tissue Processing

1. Close the sterile consumable set.
   NOTE: The isolation system (see **Table of Materials**) confirms the completeness by performing a wet test.
2. Follow the instructions on the display.
   NOTE: The process is mostly software controlled and only requires the manual confirmation for the listed steps below by pressing the buttons "next" or "back" on the control panel. The lipoaspirate is transferred into the tissue collection canister. In the tissue collection canister, the lipoaspirate is weighed and automatically cleaned with Ringer's lactate solution in order to remove the residual blood cells and wetting solution.
3. Add more fat if required.
   NOTE: Depending on the weight of harvested adipose tissue the isolation system asks the user to add more fat or it calculates the amount of additional enzyme reagent to add.
4. Add the required amount of enzyme reagent when prompted by the isolation system.
   NOTE: During the process of enzymatic digestion, separation of lipid and the SVF is achieved. Thereby, the tissue is in oscillating motion. After the enzymatic digestion is completed, the SVF is automatically transferred into the centrifuge chamber for cell concentration. In the centrifuge chamber, the SVF is concentrated to a cell pellet (centrifuge speed is approximately 3,000 x g) . Finally, the cell pellet undergoes a series of washes with Ringer's lactate solution to reduce residual enzyme levels in the output. When the process is completed, 5 mL of clear fluid (SVF) is obtained.

5. Surgical Procedure

1. To approach the neuroma, make a skin incision over the previously diagnosed neuroma site in the distal third of the forearm.
2. Under the usage of surgical binocular loupes magnifier glasses (2.5 - 3.5X), continue to dissect the subcutaneous tissue and identify the involved nerve proximal to the neuroma.
   1. Subsequently, follow the nerve distally and identify the neuroma (based on visual inspection).
3. Once the full extension of the neuroma is clear, begin to excise the neuroma by a straight trans-neural scissor cut. If no neuroma is identified, only neurolysis of the nerve is performed.
4. Continue with the neurolysis of the remaining nerve stump until 3 cm proximal to the wrist.

NOTE: The end of the nerve stump should be located in the middle of the principal incision.
5. Arrange two to four small puncture (stab) incisions (1 mm in diameter) with a scalpel through epidermal and dermal cutis around the principal approach.
6. Place two to four blunt cannulas (16-gauge, length 9 cm) via the stab incisions perineural to the stump.
   NOTE: The tip of the cannulas should be directed towards the nerve stump.
7. Secure the cannulas in place by adhesive dressing.
8. Place the stitches around the cannulas and leave the suture material in situ without securing the node.
9. Suture the main incision tightly in order to prevent the outflow of grafting.
   NOTE: Ensure that cannulas remain *in situ*.

6. Application of the SVF-enriched Fat Graft

NOTE: After approximately 1.5 h of processing, the 5 mL of the processed SVF is ready for further use.

1. Aspirate 5 mL of SVF in a 10-mL communicating syringe.
2. In the same manner, aspirate 2 mL of the sedimented lipid fraction in the opposing 10-mL syringe.
3. Mix the 5 mL of SVF with the 2 mL of the sedimented lipid fraction by alternating pulling back the plunger of one syringe and pulling down the plunger of the communicating, opposing syringe until sufficient mixing is obtained.
4. Divide the SVF-enriched fat graft equally in two to four 10 mL syringes.
5. Distribute the prepared SVF-enriched fat graft one after the other equally around the nerve stump by connecting the syringes to the blunt cannulas.
   NOTE: Before applying the graft, ensure that the cannulas are secured in the correct position and be careful not to dislocate the cannulas while applying the fat graft.
6. Remove the cannulas carefully.
7. Then, tape the small puncture incisions with steri-strips.
8. Immobilize the hand by applying a splint in the intrinsic plus position (metacarpophalangeal (MCP) joints flexed at 60 - 70°, the interphalangeal (IP) joints fully extended, thumb in the fist projection, wrist in dorsal flexion of 25°). If only neurolysis of the nerve is performed, no splint is needed and arm and wrist are bandaged for 10 days.

7. Postoperative Patient Handling

1. Transfer the patient to the post-anesthesia recovery unit post-surgery, and allow for sufficient monitoring in the ward.
2. Continue to immobilize the wrist for 10 days after the surgery.
3. Perform the first change of bandages after 48 h post-surgery and continue at regular intervals (*i.e.* every second day until discharge).
4. Remove the sutures 12 - 14 days postoperatively.
5. Perform clinical assessment of pain during follow-up at 2, 6, 12, and 36 months.

Representative Results

Data were analyzed using statistical software and presented as means ± standard deviations. The Kolmogorov-Smirnov test was applied to verify the nonparametric distribution of the study population and confirmed a negative empirical distribution. Based on the nonparametric nature of the study population, the Wilcoxon's test for related samples was employed to compare preoperative and postoperative outcomes. Statistical significance was regarded at p-values less than 0.05[10].

Five patients with a mean age of 49.8 ± 16.6 years were included in the study. The comparison of pain scores before and after the surgery are presented in **Table 1** for each pain modality and overall pain. The treatment with SVF-enriched fat grafting could not show statistically significant pain reduction. However, a relevant reduction in all pain modalities could be achieved from 2 months postoperatively onwards and showed constant with no pain relapse up to the 36 month follow-up (**Figure 1**).

Spontaneous pain could be reduced from preoperatively 1.6 ± 0.55 to postoperatively 1.2 ± 1.1 (p = 0.414), spikes from 2.2 ± 1.3 to 1.4 ± 1.34 (p = 0.180), hyperesthesia from 1.6 ± 1.14 to 1.2 ± 1.64 (p = 0.713), tap pain from 2.8 ± 0.45 to 1.8 ± 1.3 (p= 0.197) and motion pain from 2.8 ± 0.45 to 1.4 ± 1.34 (p = 0.066). Overall a pain reduction from preoperatively 2.2 ± 0.97 to postoperatively 1.4 ± 1.26 could be achieved 36 months after the surgery (p = 0.104).

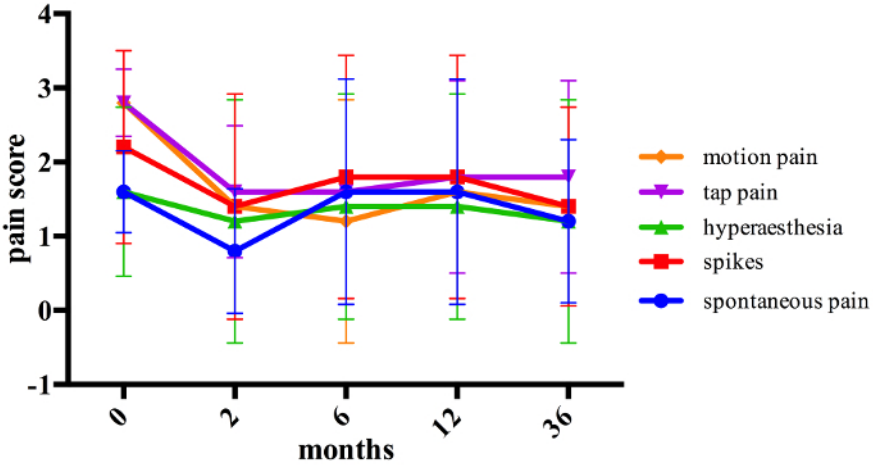

Figure 1: The trend of pain modalities. The assessment of pain modalities (mean ± SD) was performed both preoperatively and 2, 6, 12, and 36 months postoperatively.**This figure has been modified from a previous publication**[10]. Please click here to view a larger version of this figure.

Table 1. Mean pain scores ± standard deviations (p-value) before and after SVF-enriched fat grafting						
	spontaneous pain	**spikes**	**hyperesthesia**	**tap pain**	**motion pain**	**Overall pain score**
Preoperative score	1.6 ± 0.55	2.2 ± 1.3	1.6 ± 1.14	2.8 ± 0.45	2.8 ± 0.45	2.2 ± 0.97
2 months	0.8 ± 0.84 (0.102)	1.4 ± 1.52 (0.285)	1.2 ± 1.64 (0.713)	1.6 ± 0.89 (0.083)	1.4 ± 1.52 (0.102)	1.28 ± 1.24 (0.225)
6 months	1.6 ± 1.52 (1.0)	1.8 ± 1.64 (0.655)	1.4 ± 1.52 (0.713)	1.6 ± 1.52 (0.157)	1.2 ± 1.64 (0.102)	1.52 ± 1.45 (0.345)
12 months	1.6 ± 1.52 (1.0)	1.8 ± 1.64 (0.655)	1.4 ± 1.52 (0.713)	1.8 ± 1.3 (0.180)	1.6 ± 1.52 (0.109)	1.64 ± 1.38 (0.345)
36 months	1.2 ± 1.1 (0.414)	1.4 ± 1.34 (0.180)	1.2 ± 1.64 (0.713)	1.8 ± 1.3 (0.197)	1.4 ± 1.34 (0.066)	1.4 ± 1.26 (0.104)
p-value shows the difference between the pre- and postoperative scores						

Table 1: The diverse pain modalities affecting the skin over the neuroma. The diverse pain modalities affecting the skin over the neuroma were rated by our patients from 0 to 3 for each pain modality [none (0), mild (1), moderate (2), and severe pain (3)]. The clinical examination was performed prior to surgery and during the follow-up at 2, 6, 12, and 36 months post-surgery. Pain scores were postoperatively assessed over the original location of pain as well as over the site of SVF-enriched fat grafting. This has been modified from a previous publication[10].

Discussion

The purpose of the present study is to methodically illustrate the technical aspects of SVF-enriched fat grafting as a novel treatment for symptomatic end-neuromata.

Neuromata of the SBRN are associated with a surgical success rate of only 33%[12]. Vaienti *et al.* presented a series of favorable results in early follow-up using perineural fat grafting (PFG) without adding the SVF in 8 patients with painful neuromata of the upper limb[5]. However, pain reduction proved to be inconsistent after 6 months with pain relapse at the 12 month follow-up[5].

While pain reduction did not prove to be statistically significant in SVF-enriched fat grafting of the SBRN, a clear trend towards constant pain reduction was noticed in all pain modalities[10]. Moreover, the pain reduction was continuous from 2 months up to 36 months postoperatively with no pain relapse. Therefore, the clinical assessment at 2 months post-surgery seems to be a reliable predictor in terms of future long-term prognosis. However, a bigger randomized series is necessary for a detection of potential statistical significance. This favorable outcome might be seen in the context of a greater residual volume of the fat graft. Previous studies demonstrated reduced fat resorption rates when ADSC-enriched fat grafting was applied as compared to non-enriched graftings[13]. Moreover, the regeneration potential of the SVF-enriched fat graft is boosted by numerous mechanisms on the cellular level, principally improving the angiogenesis, inflammation, and fibrosis. ADSC as a component of the SVF triggers angiogenesis by the expression of growth factors like VEGF and bFGF on the cellular level[14]. The immune system is influenced by hematopoietic cells like macrophages of the M2 phenotype or T helper cells and natural killer cells[15]. Cellular signaling can be affected by a decreased number of pro-inflammatory cytokines such as TNF-α and IL-6[16]. It also has a beneficial effect on neural tissue itself by reducing unorganized nerve outgrowth and subsequent painful cutaneous scarring. Paik *et al.* proposed an ideal concentration of 10,000 cells per 200 µL of adipose tissue for optimal graft survival and mechanism of action. Higher amounts of SVF were associated with significantly increased fat resorption rates[17].

Multiple surgical techniques in the treatment of painful end-neuromata of the SBRN have been extensively published. The technique of neuroma excision and intramuscular transposition of the remaining nerve stump into the brachioradialis muscle is still popular and widely performed. SVF-enriched fat grafting compared to intramuscular transposition does not require the surgical preparation of the brachioradialis muscle and

correlates with a shorter immobilization of the wrist. Furthermore, the technique is not limited to the presented nerve population and can be applied to any neuroma in both upper or lower extremities.

Despite the valid nerve blocking that was performed before the surgery to confirm the diagnosis and exclude pain from overlapping nerves, a mutual interference cannot be absolutely excluded. The anatomical overlap of the SBRN and LACN might cause postoperative disorganized outgrowth of the LACN in the denervated innervation area. Furthermore, the present study is limited due to the small number of patients. Although the study is the largest thus far to investigate the long-term outcome and illustrate the technical aspects of SVF-enriched fat on a single nerve population, further randomized trials with larger numbers of participants are needed to confirm the favorable trend of this novel technique.

Disclosures

The authors have nothing to disclose.

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References

1. Mackinnon, S. E., Dellon, A. L. Results of treatment of recurrent dorsoradial wrist neuromas. *Ann Plast Surg.* **19** (1), 54-61 (1987).
2. Hazari, A., Elliot, D. Treatment of end-neuromas, neuromas-in-continuity and scarred nerves of the digits by proximal relocation. *J Hand Surg Br.* **29** (4), 338-350 (2004).
3. Goldstein, S. A., Sturim, H. S. Intraosseous nerve transposition for treatment of painful neuromas. *J Hand Surg Am.* **10** (2), 270-274 (1985).
4. Koch, H., Haas, F., Hubmer, M., Rappl, T., Scharnagl, E. Treatment of painful neuroma by resection and nerve stump transplantation into a vein. *Ann Plast Surg.* **51** (1), 45-50 (2003).
5. Vaienti, L., Merle, M., Battiston, B., Villani, F. Gazzola, R. Perineural fat grafting in the treatment of painful end-neuromas of the upper limb: a pilot study. *J Hand Surg Eur Vol.* **38** (1), 36-42 (2013).
6. Zuk, P. A., *et al.* Multilineage cells from human adipose tissue: implications for cell-based therapies. *Tissue Eng.* **7** (2), 211-228 (2001).
7. Fraser, J. K., *et al.* The Celution System: Automated Processing of Adipose-Derived Regenerative Cells in a Functionally Closed System. *Adv Wound Care (New Rochelle).* **3** (1), 38-45 (2014).
8. Guo, J., *et al.* Stromal vascular fraction: A regenerative reality? Part 2: Mechanisms of regenerative action. *J Plast Reconstr Aesthet Surg.* (2015).
9. Lutz, B. S., Ma, S. F., Chuang, D. C., Chan, K. H. Wei, F. C. Interposition of a pedicle fat flap significantly improves specificity of reinnervation and motor recovery after repair of transected nerves in adjacency in rats. *Plast Reconstr Surg.* **107** (1), 116-123 (2001).
10. Calcagni, M., *et al.* The novel treatment of SVF-enriched fat grafting for painful end-neuromas of superficial radial nerve. *Microsurgery.* (2016).
11. Hawker, G. A., Mian, S., Kendzerska, T. French, M. Measures of adult pain: Visual Analog Scale for Pain (VAS Pain), Numeric Rating Scale for Pain (NRS Pain), McGill Pain Questionnaire (MPQ), Short-Form McGill Pain Questionnaire (SF-MPQ), Chronic Pain Grade Scale (CPGS), Short Form-36 Bodily Pain Scale (SF-36 BPS), and Measure of Intermittent and Constant Osteoarthritis Pain (ICOAP). *Arthritis Care Res (Hoboken).* **63 Suppl 11** S240-252 (2011).
12. Stokvis, A., van der Avoort, D. J., van Neck, J. W., Hovius, S. E. Coert, J. H. Surgical management of neuroma pain: a prospective follow-up study. *Pain.* **151** (3), 862-869 (2010).
13. Kolle, S. F., *et al.* Enrichment of autologous fat grafts with ex-vivo expanded adipose tissue-derived stem cells for graft survival: a randomised placebo-controlled trial. *Lancet.* **382** (9898), 1113-1120 (2013).
14. Zhu, M., *et al.* Adipocyte regeneration after free fat transplantation: promotion by stromal vascular fraction cells. *Cell Transplant.* **24** (1), 49-62 (2015).
15. Eto, H., *et al.* Characterization of human adipose tissue-resident hematopoietic cell populations reveals a novel macrophage subpopulation with CD34 expression and mesenchymal multipotency. *Stem Cells Dev.* **22** (6), 985-997 (2013).
16. Premaratne, G. U., *et al.* Stromal vascular fraction transplantation as an alternative therapy for ischemic heart failure: anti-inflammatory role. *J Cardiothorac Surg.* **6** 43 (2011).
17. Paik, K. J., *et al.* Studies in Fat Grafting: Part V. Cell-Assisted Lipotransfer to Enhance Fat Graft Retention Is Dose Dependent. *Plast Reconstr Surg.* **136** (1), 67-75 (2015).

