

Role of Fat Graft Alone versus Enriched Fat Graft with Stromal Vascular Filtrate in Painful Amputation Stump

Abstract

Background: Traumatic amputations are very prevalent in today's world. Successful rehabilitation of an amputee largely depends on how well he/she adapt to prosthesis. However, because of poor scar characteristics, these patients often complain of pain while using prosthesis. Autologous fat graft is being vastly used all over the world to improve the scar of various etiologies. However, it has been associated with unpredictable resorption rate. **Objectives:** We report the results of the study which was done to assess the consequences of fat grafting over scars and to see its effects on pain management in amputated stump and compare the autologous fat graft with stromal vascular filtrate (SVF)-enriched fat graft for scar remodeling and pain modulation on amputation stumps. **Materials and Methods:** A prospective randomized trial was conducted from July 2014 to December 2015. A group of ten patients, who were unable to wear prosthesis due to painful amputation stump, incorporated in the study and randomly distributed in two groups. Group A of five patients (case group) was treated with autologous fat graft enriched with SVF while Group B (control group) of remaining five patients was treated with fat graft alone. The results were assessed at baseline, at 1 month, and at 6 months postoperatively using patient and observer scar assessment scale (POSAS) score. Magnetic resonance imaging (MRI) was done to compare fat content preoperatively and 6 months postoperatively. **Results:** All ten patients reported improvement in scar characteristics, most notably in pain in both scales of POSAS score. The improvement was comparable in both groups. However, the fat content in case group was significantly more in comparison to control group when assessed 6 months postoperatively using MRI scan. **Conclusion:** Autologous fat grafting is a viable and minimally invasive solution for painful amputation stump. Enrichment of fat graft with SVF can enhance its viability over long term. This study was done as a pilot project. Hence, further long term studies with large sample size are needed to ascertain the benefits observed in this study.

Keywords: Adipose-derived stem cells, amputation stump, fat grafting, stromal vascular filtrate

Introduction

Traumatic amputations are very common happening in today's world. The management of an amputee is not easy task. Because of unstable scar with poor scar characteristics, the management of amputation stump is a very challenging task. The quality of amputation stump scar has a very significant role on rehabilitation of these patients. Most of these patients suffer from pain that does not allow the consistent wearing of a prosthetic socket (This may include excessive pain, inability to achieve adequate suspension of prosthesis on an individual, continual skin breakdown, excessive pressure/shear on skin, soft tissue, nerves, scars, etc.). Thin atrophied skin cover with thin padding over underlying nerve endings may be the

most probable reason of pain. Successful rehabilitation of an amputee depends on the ability to successfully adapt to prosthesis.

A clinical solution that allows for the minimally invasive generation of new soft tissue padding at an amputation site is thus required. The processed autologous fat has been found to be effective treatment for improvement of the difficult scars, including burns and trauma.^{1,2} Autologous fat grafting may be a potential solution. It is a minimally invasive technique. It acts in two ways. Its physical effect forms a smooth gliding surface, which facilitates smooth movement of the nerves.³ It also produces biological changes at amputation stump sites which include neoangiogenesis and modulation of on-going inflammatory process and thus prevents the adherence of scar to its bed.^{4,5}

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However, the major concern associated with fat graft is its unpredictable survival on long term. The inadequate vascularity of the transplant can be responsible for this behavior of the fat graft.⁶ Recently, it has been found that adipose derived stromal cells (ADSCs) have improved the maintenance of volume of fat graft.⁷⁻⁹ The stromal vascular filtrate (SVF) is a diverse pool of cells which mainly consists of adipose-derived stem cells (ADSCs).¹⁰

It appears that till now no study has addressed the problem of painful amputation stump with fat grafting. We conducted this study as a pilot project to see the effect of a modified preparation of the fat graft (stromal cell-enriched adipose grafting), over amputation stump in terms of improvement in scar characteristics, and durability of fat graft in addition to its effect on painful amputation stump.

Materials and Methods

Study overview

The study was conducted in the Department of Plastic and Reconstructive Surgery in collaboration with the Department of Blood Transfusion Medicine and Department of Radio Diagnosis and Imaging at PGIMER Chandigarh. It was a randomized prospective study conducted from July 2014 to December 2015 including a followup period of 6 months. During this study, 10 patients with painful amputation stumps were selected randomly using envelop method.

Inclusion specifications

- More than 18 years of age
- Should be able to give informed consent
- Patients with amputated limb
- Discontinue use of prosthesis because of pain at amputation stump
- Inadequate soft tissue padding at amputation stump
- Intact skin cover at amputation stump site.

Exclusion specifications

- Less than 18 years of age
- Patient not fit to give informed consent
- Active drainage or active infection
- Patients receiving chemotherapy or radiotherapy treatment
- Known cases of coagulopathy disorders
- Pregnancy
- Diabetes mellitus
- Psychiatric patients.

Patients were incorporated in this study after obtaining informed written consent and approval from ethical committee. All patients selected for the study were assessed clinically and routine preoperative examinations were done. We have included those patients who had pain on prosthesis wearing more than 3 months. All prosthesis are not end bearing, but still they need to be snugly fit over amputation stump and need healthy padding of tissue beneath it.

Photographic records were also made for all patients. This was done before procedure and during scheduled followup visits. Magnetic resonance imaging (MRI) scans of amputation stumps of all patients were done preoperatively and 6 months postoperatively to assess the fat area over the amputation stump using specific sequence T1ax, T1cor, T2 space non-FS. These patients were divided into two groups randomly using computerized methods. One group, the case group, was treated with SVF-enriched autologous fat graft, while the second group, the control group, was treated with fat graft alone.

Scar assessment

In all patients, clinical assessment of scars was done with the patient and observer scar assessment scale (POSAS) score preoperatively and at 1 month and 6 months postoperatively.¹¹ This score is based on a numerical scale (1–10). Scar characteristics worsen as the score increases. A score of 10 suggests the worst scar characteristic, while a score of 1 suggests that scar has characteristics of normal skin.

Procedure

The autologous fat graft was harvested from the available donor sites taking into consideration of the patient's preference and natural fat/deposition, from abdomen and thighs. The whole procedure was done as a day care procedure. Epidural or general anesthesia was used for removal of fat. Ringer lactate solution with a concentration of 1:400,000 epinephrine was used as wetting solution. In all cases, 1 mL of the prepared solution was injected for each milliliter of fat to be harvested. Infiltrate was allowed a minimum of 7 min time before harvesting. Harvesting was done using liposuction cannula connected with a 10 ml syringe. The liposuction cannula was inserted through a stab incision in fat harvest site. The plunger of the 10 cc syringes was pulled back only a few milliliters during suctioning, to create minimal negative pressure in the syringe so as to minimize damage to fat globules. Incisions were closed with single interrupted nylon suture. After the 10 cc syringe was full, the cannula was disconnected from the syringe and a Luer Lock plug was used to cap the syringe. Free oil decanted off the top and/or wicked away with absorbent pads. Excess water and blood were got collected at the bottom which was removed.

The refined fat was then immediately transferred to laboratory in the Transfusion Medicine Department. The lipoaspirate was divided into two portions. One portion was utilized in isolation of the SVF with 0.075% collagenase. It was treated in buffered saline and agitation was done for ½ h at 37°C. The centrifugation at the rate of 1200 RPM for 5 min was used to separate SVF from the lipoaspirate. The SVF was found in the pellet derived from the centrifuged fat. It was found in the lower portion of the lipoaspirate. The cells of freshly isolated SVF were analyzed for viability using trypan blue dye exclusion test and total nucleated cell counts. The small portion of the supernatant of the final wash of the treated SVF was sent for culture. The second portion of lipoaspirate

was used to isolate the fat from rest of the solution with the help of syringe, which was held vertically. The isotonic saline was added to segregate fat and saline and the exudates were dispensed. This process was continued till the time fat becomes yellow and got rid of blood and other constituents of lipoaspirate. At the end, we got pure fat only. The SVF and the purified fat were poured in 10 ml syringes. These syringes loaded with the mixture were used for injection.

This final SVF fat graft syringe was used for injection into amputation stump of case group. For control group, the fat alone was used and it was processed as described above. Planned incision sites were anesthetized with 0.5% lignocaine with 1:200,000 epinephrine, and small stab incisions were made for the placement of fat through which cannula was inserted. Fat graft injections were given over thin, atrophied scar of amputation stump and/or painful area of stump. During fat grafting, deeper structure scarring was taken care by subcision followed by placement of fat. The fat was placed gently during the withdrawal of a blunt infiltration cannula. The stab incisions used to place the fat were closed with single interrupted nylon sutures.

The patients went home after the procedure same day.

Followup

The patients were followed clinically (using POSAS) to see changes in scar characteristics at 1 and 6 months and radiologically (MRI of amputation stump) to see the change in fat area around amputation stumps at 6 months after the procedure. The patients allowed to wear prosthesis after 4 weeks of fat grafting assessing the POSAS.

Results

Results were analyzed using various statistical methods. Both groups were tested for age distribution, gender distribution, various modes of injury, elective/emergency amputation, level of amputation, and amount of lipoaspirate injected. Both groups were found comparable as $P > 0.05$ in all comparisons. The age of the patients enrolled in this study range from 22 to 45 years while the mean age was 32.10 years. Among all causes of amputation, high voltage electric burns were the predominant (50%), road traffic accident was second most common (30%), while machine crush and mangled extremity as a result of fall from height were the rest causes. Among 10 subjects, upper limb was involved in 7 (70%) while lower limb was involved in rest 3 (30%). Out of 10 subjects, there were 6 (60%) cases of below elbow amputation, 2 of above knee amputation (20%), 1 of above elbow level, while 1 was of below knee level. The amount of lipoaspirate harvested in all subjects varies from 25 to 65 ml. The mean of lipoaspirate in both groups was 34 ml [Figure 1].

The cells from SVF were analyzed for viability and total nucleated cells were counted [Table 1].

In both groups, scar assessment was done using POSAS score. In both groups, POSAS score decreased over

period which shows improvement in scar characteristics [Table 2 and Figure 2].

On analyzing the patient and observer score in both case and control groups over a period of 6 months using Wilcoxon signed-ranks test, the decrease in score was found statistically significant. The $P < 0.05$ in all assessments was found. However, on statistically analyzing this result using Mann–Whitney test, a decrease in POSAS score was comparable in both case and control groups as $P > 0.05$. It signifies that autologous fat graft improves scar characteristics and addition of SVF does not make a significant difference in outcome. All the individual parameters of POSAS score were also studied and found to be decreased in value over the study period in both P -score and O -score. This signifies that all characteristics of the scar showed improvement over a period of time [Figures 3 and 4] The changes were found significant on statistical analysis ($P < 0.05$).

All patients were assessed for change in fat area over amputation stump using MRI scan of stump preoperatively and 6 months postoperatively. The specific MRI sequence T1ax, T1cor, T2 space non-FS was used in all patients to assess fat area at amputation stump preoperatively and 6 months postoperatively. The same cut-section of MRI was used to assess in preoperative and postoperative time to maintain uniformity. There was increase in fat area in all patients on MRI scans 6 months postoperatively. In case group, the mean fat area preoperatively was 17.88 which changed to 26.82 at 6 months postoperatively, while in control group, the preoperative fat area was 24.07 which changed to 28.76 at 6 months postoperatively [Figure 5]. Overall, in all 10 patients, the mean fat area preoperatively was 20.97 which changed to 27.79 at 6 months postoperatively.

On statistically analyzing this result using paired sample statistics, the change in fat area in case group ($n = 5$) was found significant. The $P = 0.009$ (< 0.05) was found. However, in control group ($n = 5$), the change in fat area was not found significant as the $P = 0.158$ (> 0.05).

MRIs were taken preoperatively and at 6 months postoperatively to assess change in fat area objectively. Comparison of fat in both images was done at same level and same plane using fixed points Figures 6 and 7.

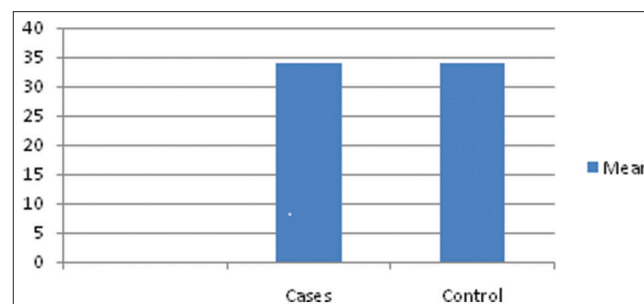


Figure 1: Mean of quantity of lipoaspirate in both groups (mL)

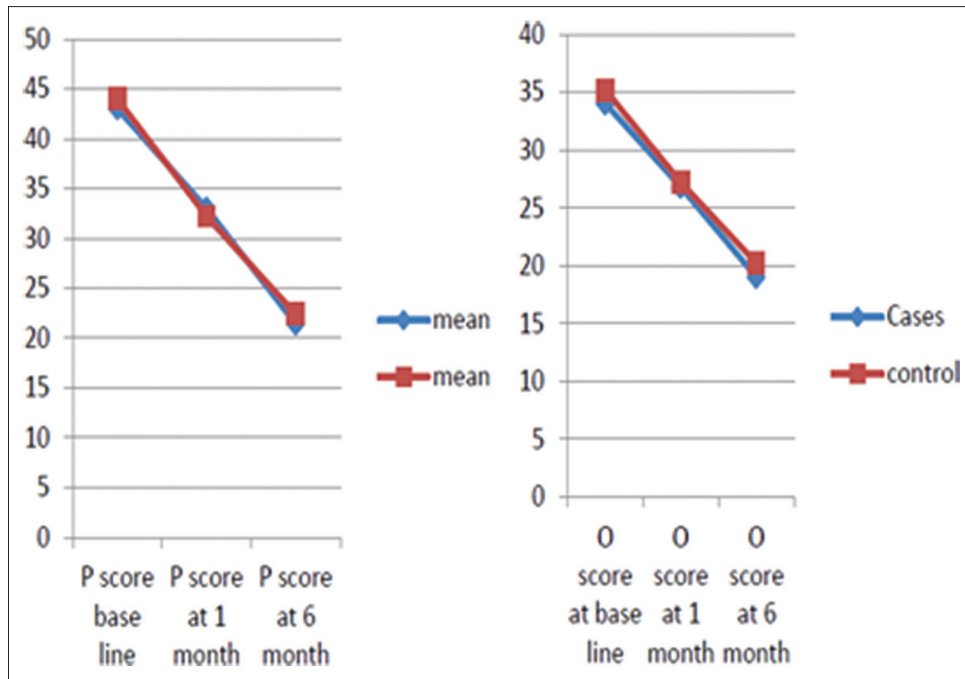


Figure 2: Decreasing trends in patient and observer scar assessment scale score in both groups

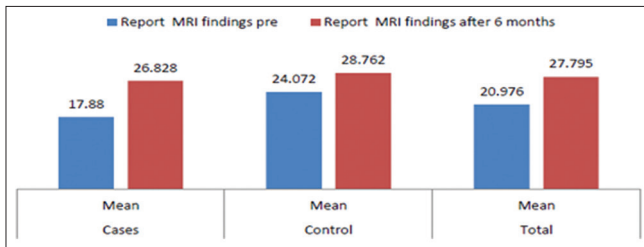


Figure 3: Diagrammatic representation of change in mean fat area in both groups

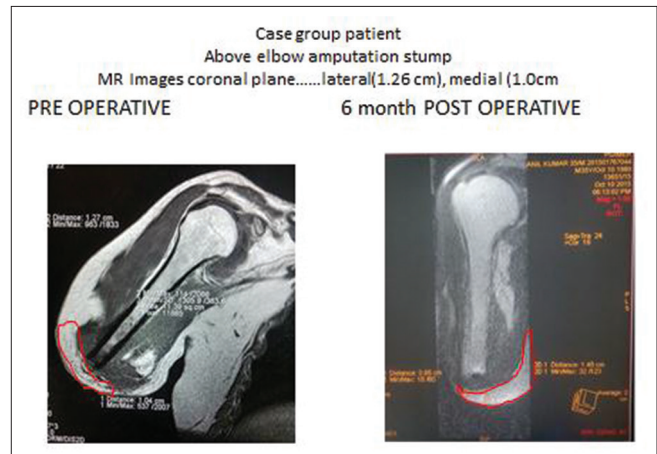


Figure 4: Magnetic resonance imaging scan of amputation stump in a case group patient showing change in fat area preoperatively and 6 months postoperatively

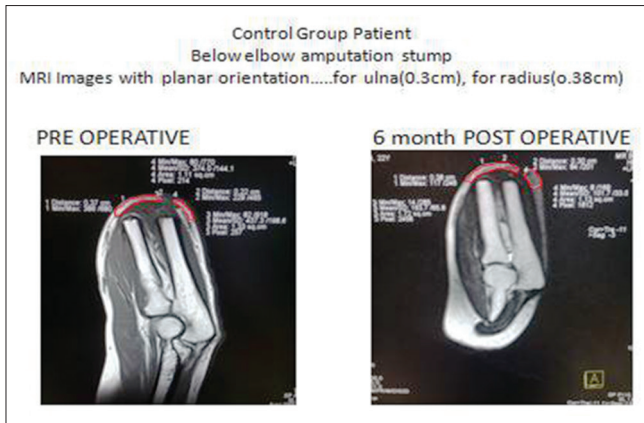


Figure 5: Magnetic resonance imaging scan of amputation stump in a control group patient showing change in fat area preoperatively and 6 months postoperatively

Discussion

The management of amputation stump is an uphill task for any physician. It is very important for rehabilitation of an amputee. Successful and early rehabilitation depends on the use of prosthesis. Most of the amputee patients complained

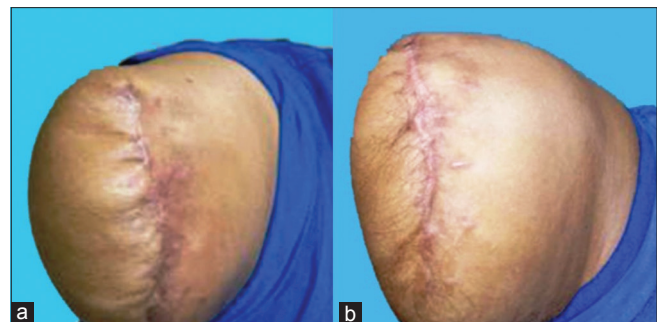


Figure 6: Preoperative (a) and 6 months postoperative (b) photographs of above elbow amputation stump showing improved scar characteristics

of pain while wearing prosthesis. Mostly, it is because of thin soft tissue cover over the bony structures. It leads to

Table 1: Change in the patient and observer scar assessment scale score (Mean)

Group	P Score Base Line	P Score at 1 Month	P Score at 6 Month	O Score Base Line	O Score at 1 Month	O Score at 6 Month
Case	43	33	21.40	34	26.80	19
Control	44	33.2	22.40	35.20	27.20	20.20

Table 2: Change in mean fat area preoperative and 6 months postoperative in both groups

Group	MRI Findings Pre operatively	MRI findings postoperative after 6 months
Case	17.8800	26.8280
Control	24.0720	28.7620
Total	20.9760	27.7950



Figure 7: Preoperative (a) and 6 months postoperative (b) photographs of below knee amputation stump showing improved scar characteristics

poor socket interface between stump and prosthesis, which further compounds by recurrent ulceration. In the past, it used to be treated with surgical revision or a distant muscle flap over the amputation stump. The further surgery at amputation stumps leads to shortening of the bone, lengthy scars, and prolonged healing. Furthermore, these procedures are highly invasive and add to the morbidity of the patient. A clinical solution that allows generation of new soft tissue padding at an amputation site and also offers relief without further limb shortening, lengthy scars, prolonged recovery, and significant donor site morbidity is required.

Autologous fat grafting is a reassuring and potent therapeutic solution to scars of varied origins. It is being done commonly to provide bulk and to correct skin contour deformities. Autologous fat grafting can improve scar quality. Klinge *et al.* conducted a study on painful and retractile scars all over the body in 694 patients over a period of 6 years with different etiologies.¹² They showed improvement in various characteristics of scar including pain using autologous fat grafting. Vaienti *et al.* also conducted a study on treatment of painful neuroma of upper limbs using perineural fat grafting.¹³ They recorded a mean pain reduction of around 23% and also showed improvements in the limb functionality around 18%.

The adipose tissue has important mechanical and biological properties. The adipose tissue has cells such

as preadipocytes, multipotent regenerative cells, and growth factors, which are responsible for its biological properties. Mechanically, it provides a gliding surface over stump. By virtue of its biological properties, it helps in neoangiogenesis and pain modulation of amputation stump.

However, autologous fat transplantation has a significant disadvantage. The transplanted adipose tissue got resorbed at an arbitrary rate. Grafted fat resorbs at a rate that varies from 20% to 90% most evidently in the first 6 months.^{14,15} It leads to loss of beneficial effects of fat grafting therapy. Research has been going on to device the techniques and methods to enhance the life of fat graft. The primary center of attention till now has been to standardize the procedure of fat grafting and fat cell processing, but these methods are not found to be very useful.^{16,17}

Recent research has advocated that supplementation of adipose-derived stromal cells (ADSCs), in the fat grafts, has improved volume maintenance and these enriched fat grafts are also found to have improved and better histological appearance than traditional fat grafts.⁷⁻⁹ The attention of clinicians has shifted to supplement the fat graft with adipose-derived stem cells. The SVF is a miscellaneous pool of cells, which mainly consists of adipose-derived stem cells (ADSCs).¹⁰ The SVF can be a good source of ADSCs.¹⁸ These stromal cells act by different mechanism; they improve revascularization, reduce apoptosis, and promote the differentiation of preadipocytes.

The role of autologous fat grafting in the improvement of scar quality is clinically proven, but scanty studies have been done to see its effects on modulation of pain.¹⁹ There is no published study available on use of autologous fat graft over amputation stump. However, a few clinical trials are going on whose results are not available. A pilot study is being done by Rubin at University of Pittsburgh to see the effect of injection of autologous fat at the amputation stump sites. They are also assessing the effect of autologous fat injections on pain modulation at amputation stumps.²⁰ They hypothesize that autologous fat grafting can provide a therapy which is not very invasive and can help alleviate pain syndrome at amputation stumps, by improving the amount of subcutaneous tissue padding.

We did a study to see the effect of autologous fat grafting over pain and scar remodeling at amputation stump scar. All patients were assessed using POSAS score preoperatively and at 1 and 6 months postoperatively.¹¹ All characteristics of scar show improvement as evident by change in POSAS score [Table 1 and Figure 2]. All of our patients showed

improvement in scar characteristics [Figures 6 and 7] and started using prosthesis within 6 months postoperative period. Like other studies done by Klinge *et al.*, the results of our study also suggest that autologous fat grafting over amputation stump mitigates symptoms of patients including pain.¹²

We also studied the outcome of addition of adipose-derived stem cell (SVF) on long term retention of fat graft [Figure 8]. The addition of ADSCs has effect on improved volume maintenance of fat graft as suggested by Yoshimura *et al.*⁸ In our study, the cells from SVF were analyzed for viability and total nucleated cells were counted. The mean count of cells in all our patients was 2.98×10^7 [Table 3]. The outcomes of this study also indicate that enrichment of fat graft with ADSCs has its impact on survival of fat cells and volume maintenance. MRI can be very helpful to get an insight into the long term behavior of transferred fat. These can also be helpful in understanding the processes responsible for resorption of transplanted fat.²¹ In our study, we compare the fat area on amputation stump at the end of 6 months using MRI scan [Table 2 and Figure 3]. The case group

which was treated with enriched fat graft has statically significantly more fat area at the end of 6 months. Hence, autologous fat grafting over amputation stump can be a potential treatment option for painful amputation stumps [Figures 9-10]. Further, enriching the autologous fat graft with ADSCs can improve the resorption rate of fat graft.

However, as this study was done as a pilot project, more studies with larger sample size and with long followup period are required to draw a definitive conclusion. The other limitation of this study was that we studied the change in area instead of volumetric assessment of fat at amputation stump as the necessary software was unavailable. Further studies are also required to see the effect of use of prosthesis on long term benefits of fat transplantation on amputation stumps.

Conclusion

Autologous fat grafting is a viable and minimally invasive solution for painful amputation stump. The SVF is a pool of different cells which mainly consists of ADSCs. These cells

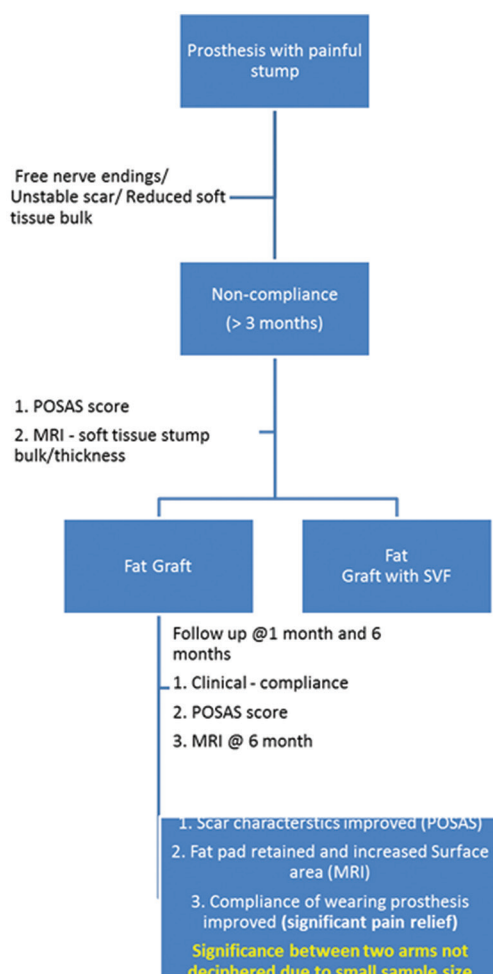


Figure 8: Algorithm showing study design

Table 3: Total nucleated cell count in stromal vascular filtrate

Group	Minimum	Maximum	Mean	Standard deviation
Case	2.50×10^7	3.70×10^7	2.98×10^7	0.57619



Figure 9: Preoperative, 6 months postoperative & 18 months postoperative photographs of below knee amputation stump showing improved scar characteristics



Figure 10: Preoperative, 6 months postoperative & 18 months postoperative photographs above elbow amputation stump showing improved scar characteristics

improve fat graft survival through various mechanisms. Which include improvement in revascularization of fat graft, decreasing the rate of apoptosis, and boosting differentiation of adipocytes. The retention rate of fat graft is significantly improved using SVF. However, long term followup and more studies with more number of subjects are required to establish fat grafting as treatment of painful amputation stump.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patients have given their consent for their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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