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Does vascularized adipose tissue enhance nerve regeneration? An experimental study on the sciatic nerve in rats

Bogdan Ioncioaia[1], Andrei-Nicolae Coseriu[2], Alex-Victor Oradan[3], Luciana-Madalina Gherman[4], Dan Gheban[5]

[1] Plastic Surgery and Burns Division, Clinical Department of General Surgery No.1, Clinical Emergency Hospital, Cluj-Napoca, ROMANIA
[2] Plastic Surgery Department, Clinical Rehabilitation Hospital, Cluj-Napoca, ROMANIA
[3] Plastic Surgery Department, Clinical Rehabilitation Hospital, Cluj-Napoca, ROMANIA;
University of Medicine and Pharmacy "Iuliu Hatieganu", Cluj-Napoca, ROMANIA
[4] Experimental Centre of University of Medicine and Pharmacy "Iuliu Hatieganu", Cluj-Napoca, ROMANIA
[5] Department of Pathology, University of Medicine and Pharmacy "Iuliu Hatieganu", Cluj-Napoca, ROMANIA

ABSTRACT

Background. The use of adipose tissue in reconstructive procedures has become popular in the last decades due to the adipose tissue properties of stem cells and mechanical protection. In acute nerve lesions, there have been experimental reports on the use of non-vascularised adipose tissue as an adjuvant for nerve recovery but to our knowledge there is no published research that describes the effect of the pedicled vascularised adipose tissue (pVAT) on acute nerve injuries. Therefore we decided to study the effect of pVAT on nerve regeneration when wrapped around an epineurial coaptation site and for a 5 mm defect of the sciatic nerve from the rat.

Methods. The effect of pedicled vascularized adipose tissue (pVAT) on nerve regeneration was studied on the sciatic nerve injury model in twenty-four Wistar rats divided into four groups: epineurial repair; epineurial repair and pVAT; a 5 mm nerve gap bridged by an autograft; a 5 mm nerve gap bridged by a conduit-pVAT (C-pVAT). Automatization injuries, walking track analysis, postoperative extraneural fibrosis and histological analysis were performed three months after the intervention.

Results. Although histological and functional nerve regeneration was present in various degrees in all the studied groups, nerve regeneration was not enhanced by the use of the pVAT or C-pVAT. The harvesting of the pVAT flap caused postoperative fibrosis when used as a conduit. Automutilation was not decreased by the use of the pVAT nor a correlation between automutilation and postoperative fibrosis in the studied groups could be established. The postoperative fibrosis did not influence neural regeneration in the pVAT and autograft group.

Conclusion. Vascularized adipose tissue did not enhance neural regeneration when used as an adjunct procedure for primary nerve repair and or even as a novel conduit for 5 mm nerve defects in the rat sciatic nerve.

Keywords
adipose flap,
nerve regeneration,
pedicled adipose transfer,
nerve repair

Corresponding author:
Bogdan Ioncioaia

Clinical Emergency Hospital,
Cluj-Napoca, Romania

bogdanioncioaia@gmail.com

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Introduction

The use of adipose tissue in reconstructive procedures has become popular in the last decades due to the adipose' tissue properties of stem cells import and mechanical protection. Adipose tissue was successfully used in cases of recurrent compressive neuropathies as a graft[1], as a pedicled flap[2] or even as free tissue transfer[3].

Vascularized tissue has the advantage of carrying its own blood supply thus being independent from the state of the recipient site providing in theory a faster therapeutical response. Depending upon the size of the gap between the ending parts of a severed nerve, the surgical treatment of acute nerve injuries consists of end-to-end coaptation, nerve grafting, nerve conduits or nerve transfer for cases where nerve regeneration takes longer to reach the motor end-plate[4].

In acute nerve lesions there has been experimental reports on the use of non-vascularized adipose tissue (fat graft) as an adjuvant for nerve recovery[5,6] but to our knowledge there is no published research that describes the effect of the pedicled vascularized adipose tissue (pVAT) on acute nerve injuries. Therefore, we decided to study the effect of pVAT on nerve regeneration when wrapped around an epineurial coaptation site and for a 5 mm defect of the sciatic nerve from the rat.

Material and method

This study was performed in line with the principles charta of animal's care and use. Approval was granted by the Ethics Committee of University of Medicine and Pharmacy Iuliu Haţieganu (23.02.2021/251).

Twenty-four Wistar rats with an average weight of 300 g were divided randomly into four groups (n=6) as seen in table 1: group 1- transection and epineurial coaptation of the nerve (N) ; group 2 – transection, epineurial coaptation and wrapping of the coaptation site with pVAT ; group 3 – nerve defect of 5 mm length and defect bridging with nerve autograft from the contralateral sciatic nerve (A); group 4 – nerve defect of 5 mm length and defect bridging with a p-VAT conduit (C-pVAT).

Anesthesia was given intramuscular (40-90 mg/kg ketamine and 5-10 mcg/kg xylazine) before the commencement of the procedure. After preparation of the incision site (hair trimming and Betadine cleansing of the skin) an intermuscular approach was chosen for sciatic nerve exposure. The sciatic nerve was exposed over its entire length in the hindlimb starting from its proximal entry location until its distal bifurcation point around the knee area (Figure 1).

Table 1. Distribution of the animal subjects into four study groups.

Group 1 N	**Group 2 pVAT**	**Group 3 A**	**Group 4 C-pVAT**
(n=6)	**(n=6)**	**(n=6)**	**(n=6)**
Transection + epineurial coaptation	Transection + epineurial coaptation + pVAT	5 mm nerve defect + Autograft	5 mm nerve defect + C-pVAT

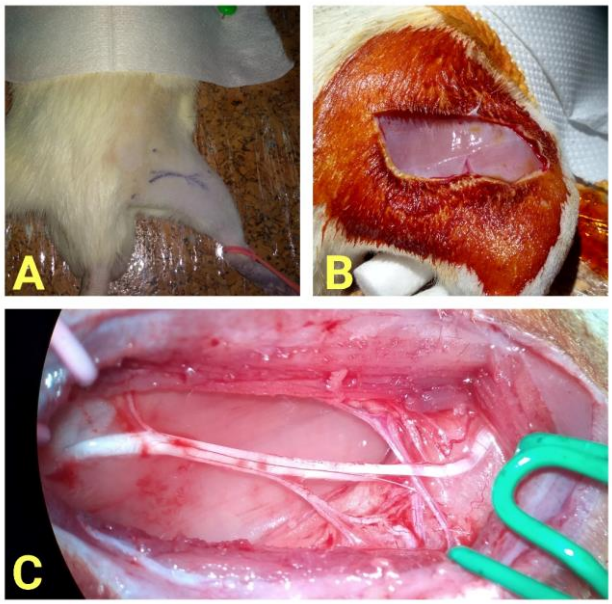

Figure 1. Preparation of the incision site and exposure of the sciatic nerve.

A. The approach for the sciatic nerve is marked having the rat placed in a dorsal position.

B. Exposure of the intermuscular septum after skin preparation.

C. Exposure of the sciatic nerve through an intermuscular approach. The left of the image depicts the proximal part of the sciatic nerve while the right part of image depicts the distal sciatic nerve with its bifurcation around the knee area.

The pVAT was harvested from the vicinity of the sciatic nerve either anterior or posterior to it using a microsurgical dissection technique with the aid of an

operating microscope. The dissected pVAT was mobilized in order to cover the recipient site without tension. Neural coaptation and pVAT wrapping was achieved with 9-0 or 10-0 nylon sutures (Figure 2).

Table 2. Scoring system used for the postneurotomy lesions.

Lesions	Score
No lesions	1
One finger injured	2
Two fingers injured	3
Three fingers injured	4
Four fingers injured	5
Five fingers injured	6

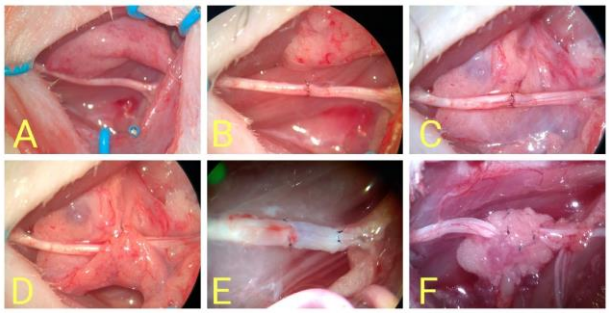

Figure 2. Various procedures performed on the sciatic nerve.
A. Mobilization of the adipose flap (pVAT) superior (anterior) to the uninjured sciatic nerve.
B. Epineurial coaptation of the sciatic nerve with the pVAT on "stand-by".
C and D. Wrapping the the pVAT around the coaptation site.
E. Autograft for 5 mm defect from the contralateral hindlimb.
F. Wrapping the pVAT as a conduit for a 5 mm defect using an inferior (posterior) approach.

After the conclusion of the procedure the rats were housed in appropriate conditions, with access to food and water ad libitum following a circadian rhythm with daily visits of the veterinary personnel. After a period of 3 months, the operated limb was examined for automutilation injuries which were and graded according to the criteria displayed in table 2, subjected to walking track analysis (WTA) followed by intraoperative nerve inspection under general anesthesia for postoperative extraneural fibrosis (table 3), concluding with a nerve sampling distal to the manipulated area of the nerve for histologic analysis using microscopy under haematoxylin eosine and trichrome Masson staining. Histological regeneration was quantified using a two-step approach. First the presence of axons distal to the procedural site was sought and their presence were suggestive for positive regeneration. Secondly the degree of regeneration was graded using a qualitative scale established by the authors: absent, weak, moderate, good (table 4) using the average values of each studied group. The WTA values were processed in order to obtain the sciatic functional index (SFI), which was further used to quantify the nerve regeneration in a qualitative manner (table 5) based on a modification of the scale used by Amniattalab et al[7].

At the end of the experiment the Wistar rats were euthanasized according to the experimental protocol. The collected data was processed and statistically analyzed using Wilcoxon matched pair test and Pearson correlation test. Experimental results were displayed as percentage or/and as mean ± standard deviation (SD); differences were considered significant when $P < 0.05$ and correlation (R) could be established (negative, absent and positive for R negative, R null and R positive values).

Table 3. Postoperative extraneural fibrosis severity scale (after Petersen et al, 1996).

Scale	Definition
1	No dissection required/ mild blunt dissection
2	Vigorous blunt dissection required
3	Sharp dissection required

Table 4. Qualitative scale of histological nerve regeneration were regeneration was present.

Average value interval	Significance
Less than 0.25	Absent
0.25 – 0.50	Weak
0.51 – 0.75	Moderate
0.76 – 1.00	Good

Table 5. Qualitative scale of functional nerve regeneration based on SFI values interval.

Sciatic Functional Index	Significance
0 to - 25	Very good
-25 to -50	Good
-50 to -75	Moderate

-75 to -99	Weak
Greater than - 99	Absent

Results

Results are displayed in table 6 and 7. Histological nerve regeneration was present in various degrees in all the studied groups (Figure 3): group 1 – moderate regeneration, group 2 – weak regeneration, group 3 – good regeneration and 4 – moderate regeneration, but neural regeneration was not enhanced by the use of the pVAT (p=0.31) neither by the C-pVAT (p=0.56). Functional nerve regeneration quantified through the SFI was deemed as good for the coaptation (-42.11±29.70), moderate for the pVAT group (-52.36±34.36), while the autograft (-85.11±13.32) and C-pVAT group (-76.11±19.17) exhibited poor regeneration. Both pVAT (p=0.52) and C-pVAT (p=-1.15) group did not enhance the regeneration of the sciatic nerve.

Postoperative extraneural fibrosis (Figure 4) was present in each of the studied groups, but to a lesser degree in group 1 and 2 (p=0.04) compared to groups 3 and 4 (p=0.05). Postoperative extraneural fibrosis in the C-pVAT group had a negative effect on functional muscle regeneration (R=0.96, P=0.02).

Automutilation was limited to one finger and was not decreased by the use of the pVAT (p>0.05) neither a correlation between automutilation and postoperative fibrosis in the studied groups could be established (p>0.05).

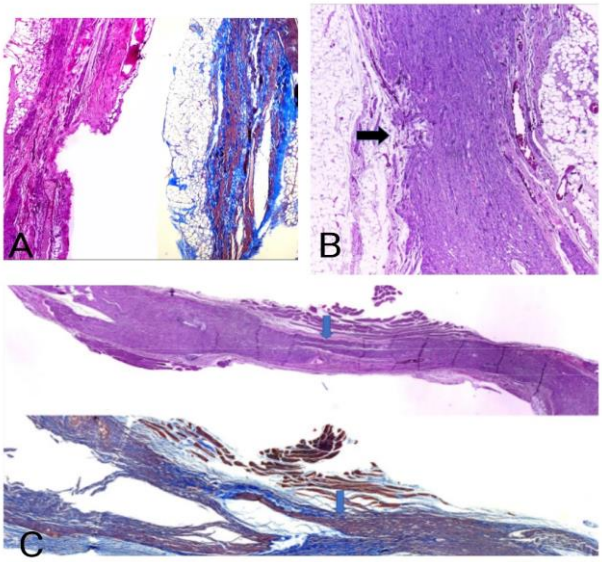

Figure 3. Histological analysis of the nerve samples collected at the repair zone depicting various degress of nerve regeneration (hematoxylin eosine and Masson trichrome staining).
A. Nerve fibers crossing the coaptation site in subject no.1 from group 1.
B. Nerve fibers display continuity in subject no. 7 from group 2.
C. Thin nerve fibers displayed in continuity in subject no. 13 from group 3.

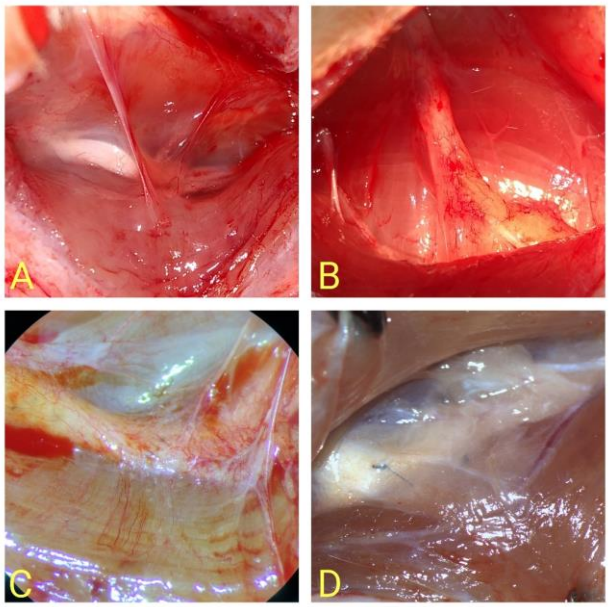

Figure 4. Various degrees of postoperative perineurial fibrosis after Petersens' classification:
A. Group 1 – Grade 1 fibrosis: mild dissection required;
B. Group 2 - Grade 2 fibrosis: vigorous blunt dissection required;
C. Group 3 – Grade 3 fibrosis: sharp dissection required;
D. Group 4 - Grade 3 fibrosis: sharp dissection required.

Table 6. Statistical analysis of the studied groups.

Postneurotomy lesions	**Mean ± SD**	**P value**
Group 1	1.33 ± 0.81	0.26
Group 2	2.16±1.47	0.71
Group 3	2.83± 2.04	0.10
Group 4	1.16±0.40	0.65
Postoperative extraneural fibrosis		
Group 1	2.0±0.63	0.04
Group 2	1.83±0.75	
Group 3	2.66±0.51	0.05
Group 4	2.66±0.51	
Nerve regeneration		
Group 1	0.66±0.51	0.31

Group 2	0.50±0.54	
Group 3	0.83±0.4	0.56
Group 4	0.66 ±0.51	
WTA		
Group 1	-42.11±29.70	0.52
Group 2	-52.36±34.36	
Group 3	-85.11±13.32	-1.15
Group 4	-76.11±19.17	

Table 7. Statistical correlation between extraneural fibrosis, automutilation, histological nerve regeneration and functional muscle regeneration.

	Fibrosis and automutilation	**Fibrosis and histological regeneration**	**Fibrosis and functional muscle regeneration**
Group 1	R=0; P=1	R=0; P=1	R=-0.27; P=0.60
Group 2	R=0.03; P=0.95	R=- 0.24; P=0.64	R=-0.17; P=0.74
Group 3	R=0.31;P=0.54	R=- 0.31; P=0.54	R=0.05; P=0.92
Group 4	R=0.31; P=0.54	R=0.25; P=0.63	R=0.96; P=0.02

Discussion

Owing it not only to the presence of adipose-derived stem cells (ASC) and its growth factors[8,9], but also to its mechanical properties adipose tissue serves as a promising add-on to the reconstructive armamentarium[10] with beneficial results in treating chronic nerve lesions[1-3]. The experimental work of Tuncel et al concluded that autologous fat graft improved the regeneration of the sciatic nerve in rats when used as an adjunct to primary coaptation, autograft and venous conduit filled with fresh or denaturated skeletal muscle[6]. Considering the reported[11] broad spectrum of fat graft take (between 20 and 80%), increased survival depends upon the optimal blood supply of the adipose tissue, which is ideal as vascularized tissue (pedicled or free tissue transfer). The vascularized adipose tissue transfer settles the issue of donor tissue preparation as in fat graft harvesting and recipient site viability, but requires careful flap dissection, maintaining the nutrient vessel in its substance. VAT in the form of pedicled or free fat flaps are being used in chronic peripheral nerve injuries with good results[1-3].

In our experiment we studied whether pVAT had a positive influence on the regeneration of an acute nerve injury model on the sciatic nerve after epineurial coaptation following complete transection and as a novel adipose conduit for a 5 mm nerve gap (C-pVAT) and its influence on postoperative fibrosis and automutilation injuries.

The p-VAT plays a similar role to the nerve wrapping procedures used in chronic neuropathies[2,3]. The C-pVAT on the other hand is a novel procedure used for small nerve gaps (5 mm) and is designed as a vascularized conduit using a similar technique as for nerve wrapping except its proximal and distal ends are secured to the nerve stumps in such a way that a tube which should facilitate the transition of axons to the target area is created. It is a technically demanding procedure that requires a patent lumen while wrapping the adipose flap around the defect and may finally cause the obstruction of regenerating nerve fibers due to its bulkiness, as was noticed in some cases.

Histological nerve regeneration was established in a qualitative manner by localizing the presence of axons in the distal stump and assigning a grade to the average value of each of the studied group in order to estimate the quality of nerve regeneration. Despite the fact that axons could be noticed distal to the procedural stump in various degrees, the use of VAT did not enhance nerve regeneration. As a caveat to the applied method we mention that the quantity of axons measured distally was not appreciated, thus the exact number of axons could not be estimated. Functional muscle regeneration was quantified by SFI values measured at 3 months postoperative that were compared to a scale developed by the authors based on other published research. The least extensive procedure presented the best outcome regarding WTA as seen in group 1 and 2 compared to group 3 and 4. Given that normal gait was compromised in the donor hindlimb for the autograft group, WTA values may have been altered and the functional nerve regeneration may have not been precisely determined in this instance.

The extent of flap dissection was direct proportional to the severity of the postoperative fibrosis (Figure 4), causing less fibrosis in the pVAT group compared to the C-pVAT group. Therefore harvesting pedicled fat from the vicinity of a nerve,

may not yield the best results for treating a nerve injury when both flap and nerve share the same location as it is known that postoperative fibrosis remains a cause of recurrrence after peripheral nerve surgery[1-3].

Automutilation of the rat's distal limb following a proximal nerve injury, indicates the loss of protective sensibility. Interestingly, the C-pVAT group exhibited fewer automutilation injuries than its counterparts, suggesting that fat may play an protective role in skin integrity during nerve regeneration although the mechanism is not understood and perhaps even play a similar role to the regenerative peripheral nerve interface for the treatment of painful neuromas[12].

Although histological and functional nerve regeneration was present in different degrees in the p-VAT and the C-pVAT groups they did not improve nerve regeneration, showing no benefit of VAT when used as an adjunct for epineurial coaptation or as a novel adipose conduit for a 5 mm nerve gap. We would like to highlight some technical aspects that may have negatively influenced our outcomes.

One limitation of our study was the difficulty in maintaining a consistent pVAT harvesting location and nerve wrapping technique so as to avoid tension and devascularization of the flap at the injury site. Although flap preparation and VAT wrapping was performed with minimal tension, after 90 days we noticed in some cases that the sciatic nerve was elongated which may have had negatively influenced the outcomes. This may have been the explanation for the poor results obtained in the pVAT and C-pVAT groups as seen in histological and functional nerve regeneration values. There was also a learning curve in the process of flap raising and insetting. The difficulty in precisely determining the main blood supply of the flap, could have made some parts of the flap non-viable, especially the distal part. Last but not least, given its volume the pVAT was difficult to tailor as an adipose conduit as to maintain a patent lumen for the advancement of nerve fibers and therefore we think that it is not suitable for bridging nerve defects in small nerves.

Conclusion

Vascularized adipose tissue did not enhance neural regeneration role when used as an adjuvant procedure for primary nerve repair or as a novel conduit for 5 mm nerve defects in the rat sciatic nerve.

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