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Evaluation of the Effects of Fat Graft Wrapping on Early Nerve Regeneration: An Experimental Study

Yağ Grefti Kullanımının Erken Dönem Sinir İyileşmesi Üzerine Etkilerinin İncelenmesi: Deneysel Bir Çalışma

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ABSTRACT Objective: It is not easy to establish ideal neural regeneration after peripheral nerve repair. Various types of microsurgical techniques, tools, pharmacological and chemical agents were used to increase the quality of the neural regeneration process. However, we are still far from the perfect neural repair. In this study; it was aimed to investigate the effect of fat graft, wrapped around sciatic nerve repair coaptation zone, in neural regeneration. **Material and Methods**: Twenty four Sprague-Dawley rats were chosen and separated into two groups equally. In the first twelve rats, skin incision was made in the right gluteal area, after blunt muscle dissection sciatic nerve was exposed. Full thickness nerve cut was performed and primary nerve repair was also performed in the same session. The first group was addressed as normal neural regeneration model. In the other twelve rats, same processes were performed in order to expose sciatic nerve. After nerve repair, fat graft harvested from the right posterior popliteal area was wrapped around the nerve coaptation zone. All the rats were sacrificed 3 weeks post-operatively, nerve segments were removed and sent for histopathological evaluation. **Results:** Our study demonstrated that macrophage migration and lymphocyte infiltration rates were significantly higher in experimental group. **Conclusion:** It was observed that wrapping fat graft around the nerve coaptation zone had a positive effect on neural regeneration by increasing macrophage and lymphocyte infiltration to the repair zone.

Keywords: Nerve regeneration; adipose tissue; peripheral nerve injuries; adipose derived stem cell

ÖZET Amaç: Periferik sinir kesisi onarımından sonra ideal sinir iyileşmesinin elde edilmesi çoğu zaman mümkün olmamaktadır. Çeşitli mikrocerrahi teknikler, aletler, farmakolojik ve kimyasal ajanlar sinir iyileşme sürecinin kalitesinin arttırılması için denenmiştir. Ancak, hala mükemmel olarak tanımlanabilecek sinir onarımından uzaktayız. Çalışmamızda rat modeli üzerinde önce kesilip sonra onarılan siyatik sinir çevresine sarılan yağ greftinin sinir iyileşmesi üzerinde olan etkileri incelendi. **Gereç ve Yöntemler:** Yirmi dört Sprague-Dawley cinsi rat seçilerek iki gruba ayrıldı. İlk grupta, sağ gluteal bölgede yapılan cilt insizyonunu takiben siyatik sinir disseke edildi. Sinir tam kat kesildikten sonra aynı seansta onarıldı. Bu ilk grup normal nöral rejenerasyon modeli olarak yerini aldı. İkinci grupta aynı işlemler yapıldıktan sonra onarım hattı çevresine popliteal bölgeden alınan yağ grefti yerleştirildi. Bütün ratlar 3 hafta sonra sinir örneklemesi sonrası sakrifiye edildi. Sinir örnekleri histopatolojik incelemeye gönderildi. **Bulgular:** Çalışmamız sonucunda onarım hatları iki grupta karşılaştırıldı ve deney grubunda makrofaj migrasyonu ve lenfosit infiltrasyonu oranlarının istatistiksel olarak anlamlı şekilde daha yüksek olduğu görüldü. **Sonuç:** Sinir onarım hattına yağ grefti sarılmasının erken dönem sinir iyileşmesi üzerine makrofaj ve lenfosit infiltrasyonunun artmasıyla pozitif bir etkisinin olduğu gözlendi.

Anahtar Kelimeler: Sinir rejenerasyonu; adipoz doku; periferik sinir yaralanmaları; adipoz kökenli kök hücre

Peripheral nerve injuries often have severe consequences on the patient's life quality. Even though peripheral nerves are one of the few mammalian tissues with the capacity for extensive regeneration and in the presence of improvements in treatment, recovery after peripheral

nerve injuries is not only often disappointing but also difficult to predict. When motor function and sensory function in the hand are altered, return to work and social life activity may be jeopardized. Considering peripheral nerve injuries are frequently located in the upper extremities and associated with a suboptimal recovery of arm and hand function, these injuries have a huge impact on socioeconomic life.[1]

The functional outcome after severe peripheral nerve injuries is unpredictable and often disappointing, although the techniques for the surgical approximation of severed nerve ends have reached a high degree of technical refinement and rehabilitation is carried out meticulously.[2]

To increase the level of nerve regeneration quality, several methods of peripheral nerve reconstruction using different materials have been investigated in the last decades to find an alternative to the most widely used methods.[3] Adipose tissue is one of the most popular areas investigated recently for peripheral nerve repair.[4,5]

There are two main reasons to explain the popularity of adipose tissue.

1) The adipose tissue is a rich source of multipotent mesenchymal cells called adipose-derived stem cells and there are numerous studies showing the positive effect of adipose-derived stem cells on nerve healing.[6,7]

2) Adipose tissue has an unique advantages of being harvested easily, cheaply, safely and with a conventional method.[8]

There are several studies in the literature that shows the positive effect of adipose-delivered stem cells in nerve regeneration.[5-9] To our knowledge, there are no data showing the effect of wrapping around fat graft to nerve coaptation zone. For that reason, we designed a study on a rat model to investigate the effect of fat graft wrapping on nerve regeneration in early nerve repair.

MATERIAL AND METHODS

All animals in this study were used according to a protocol approved by the Uludag University Ethical Committe. The animals were kept in individual cages with free access to food and water and under alternating 12-hour periods of light and darkness. Twenty four female Spradue-Dawley rats weighting between 300 and 400 g randomized into 2 experimental groups.

The study was conducted with a desicion of initial committe and the serial number of the desicion is 2012-14/02 at the date of 18.12.2012. This study has been performed in accordance with the ethical standards set forth in the 1964 Declaration of Helsinki and its later amendments.

SURGICAL PROCEDURE

All animals were anesthetized by sevofluran (Sojourn, Piramal) 250 via an inhaler. Through a 3-cm-long skin incision over the gluteal region, the right sciatic nerve, from the sciatic notch to thetrifurcation, was exposed (Figures 1, 2).

Group I (n=12): The sciatic nerve on the right side was sectioned and repaired using 7-0 interrupted polypropylene suture placed in the epineurium.

Group II (n=12): The sciatic nerve on the right side was sectioned and repaired in the same manner. 1x1 cm fat graft harvested from right side popliteal fossa, wrapped around coaptation zone (Figure, 3, 4).

Skin incisions were sutured using 4-0 polypropylene. The animals were caged separately and observed daily. 3 weeks post operatively, the animals were euthanized and the right sciatic nerves were removed. All the specimens were sent for histopathological analysis. They all were processed and examined by a single histopatholo-

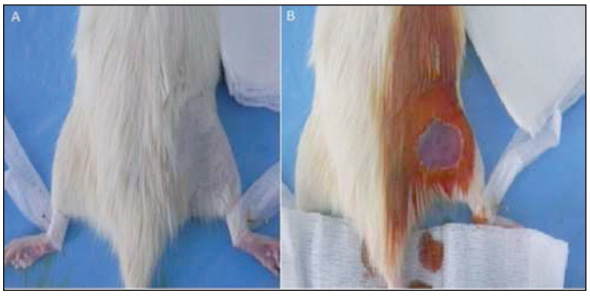

FIGURE 1: 3 cm incision over right gluteal region.

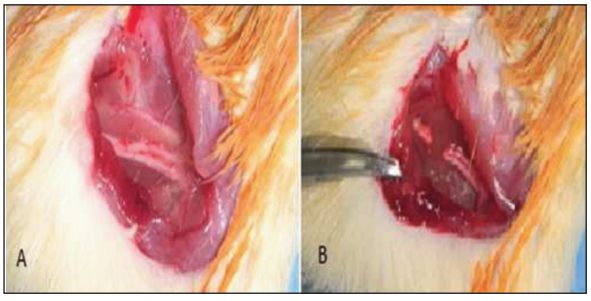

FIGURE 2: a) Right sciatic nerve **b)** Total laceration of the sciatic nerve.

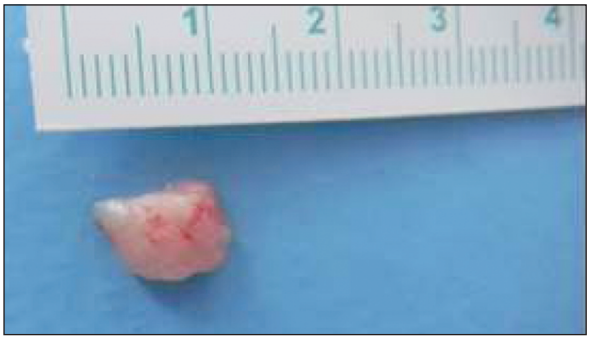

FIGURE 3: 1cm x 1cm fat graft harvested from right popliteal fossa.

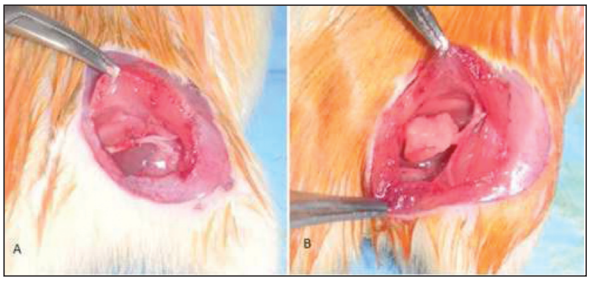

FIGURE 4: a) Epineural nerve repair, **b)** Wrapping by the fat graft.

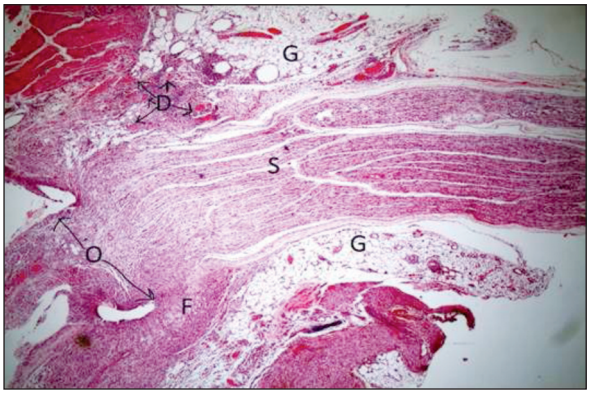

FIGURE 5: Nerve tissue under microscopic view **O:** Repair zone; **D:** Neovascularisation zones; **F:** Fibrosis; **G:** Granulation zone; **S:** Nerve body (H&E ×40).

gist. Samples were stained with Hematoxylin and Eosin (H&E), as well as Masson's Trichrome, and examined by light microscope (Figure 5). The specimens were evaluated according to lymphocyte migration, macrophage migration, neovascularitation, fibrosis formation, granulation and axonal degeneration. All statistical data were classified according to the Peterson classification system and direct cell counts.[9] All statistical data were evaluated by using SPSS program. Mann-Whitney U test was in order to investigate statistical significance. The values $p<0.05$ was considered as significantly different.

RESULTS

The fibrosis formation after nerve coaptation was investigated both for control and experimental groups. There was no significant difference between these two groups ($p=0.812$, $p>0.05$, Mann-Whitney U test).

The granulation formation was evaluated for both groups. Even though in experimental group the granulation rate was higher, there was no significant difference between two groups ($p=0.521$, $p>0.05$, Mann-Whitney U test).

The axonal degeneration rate was evaluated for both groups. There was no significant difference between two groups statistically ($p=0.806$, $p>0.05$, Mann-Whitney U test).

The neovascularization rate was evaluated for both groups. Even though the rate was higher in the experimental group. This difference was not proved statistically ($p=0.105$, $p>0.05$, Mann-Whitney U).

Macrophage migrations in the coaptation zones were evaluated for both groups. Macrophage migration was significantly higher in the experimental group ($p<0.05$, Mann-Whitney U test).

Lymphocyte infiltration rates were evaluated for both groups. Lymphocyte infiltration was significantly higher in the experimental group ($p<0.05$, Mann-Whitney U test).

The list of histopathological findings was shown in Table 1.

DISCUSSION

Epineural scar formation is an undesirable and unpredictable circumstance that directly effects the

TABLE 1: Histopathological findings.

Values	Group I (Control)	Group II (Experiment)	p
Neovascularization	10.5 (6-30)	18.5 (9-32)	0.104, p>0.05
PNL	9 (2-24)	14 (5-36)	0.064, p>0.05
Macrophage	3.5 (0-10)	11 (4-35)	**p<0.05**
Lymphocyte	24 (10-48)	82 (52-160)	**p<0.05**
Myelin vacuolization	6 (2-24)	7.5 (3-26)	0.444, p>0.05
Fibrosis	1 (1-3)	1 (1-2)	0.812, p>0.05
Inflammation	1(1-3)	2(1-2)	0.521, p>0.05
Axonal degeneration	1(1-3)	1(1-2)	0.806, p>0.05

PNL: Polimorph nuclear leukocytes.

quality of nerve regeneration. Several surgical techniques, pharmacological agent and chemical substances were applied in order to prevent epineural scar formation but we are still far from the perfect nerve regeneration. There are several publications in the literature investigating the effect of adipose derived stem cell on neural regeneration. In these publications, the positive effect of adipose derived stem cells in neural regeneration were addressed.[4,5]

An experimental study was designed in order to compare and differentiate the neural regeneration process between fat graft wrapped neural coaptation zone normally repaired coaptation zone in rat model. Two groups were formed as an experimental and control. In the control group, sciatic nerve dissected, cut in transverse pattern and coaptated. Three weeks after coaptation, coaptated nerve segment resected and was sent for histopathological evaluation. In the experimental group, sciatic nerve dissected, cut in transverse pattern, coaptated and a fat graft, harvested from popliteal fossa, wrapped around. Three weeks after coaptation, coaptated nerve segment resected and was sent to histopathological evaluation as well. The rates of fibrosis formation, inflammatory changes and regeneration rates were analyzed and classified based on patterson system.[9] Analyzed and calculated values were different from each other, hence there was no significant difference statistically.

The cells playing major roles during the neural regeneration process and revascularization rate were also counted and calculated such as polimorph nuclear leukocytes (PNL), lymphocytes, macrophages, neovascularitation sites, demyelinization sites. The experimental group had the higher numbers and rates for all variations. But only lymphocytes and macrophages were significantly different from control group ($p<0.05$).

In normal neural regeneration process, the macrophage is also the dominant cell in early healing period. It is known that macrophage has major roles such as phagocytosis of myelin debris, secretion of prolipherative process mediators and initial growth factors.[10,11] In our study macrophage infiltration rate was significantly higher in the experimental group than the control group. We consider that regeneration process were more advance in experimental group (Figure 6).

Lymphocytes are the latest acute phase cells migrating to the neural regeneration zone during early neural regeneration period. The regulation of nerve regeneration by secreting cytokines, chemokins and neurotrophins is lymphocytes' major role during early neural regeneration. Supporting macrophages during early regeneration phase to long term regeneration phase is their another important function.[12] In our study lymphocyte infiltration was significantly higher in experiment group (Figure 7).

Recent publications has highlighted the unique role of stem cells in neovascularization following injury because of their characteristics.[13,14] Eventhough there was not a significant neovascularization in our study model, it is possible to relate this result to the nature of the early neural regeneration period. It is possible to assume that neovasvularization might increase in the late neural regeneration period. In order to prove that initial studies are needed for the late neural regeneration period.

CONCLUSION

In our study, we intend to investigate the effect of fat graft on early neural regeneration. Fat graft is an autolog, easy to harvest substance. Harvesting

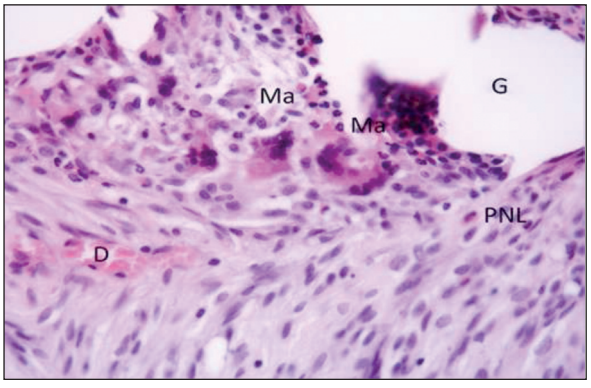

FIGURE 6: Experiment group. Nerve section. **Ma:** Macrophage migration zones; **D:** Neovascularization zones; **G:** Granulation zones (H&E ×200). **PNL:** Polimorph nuclear leukocytes.

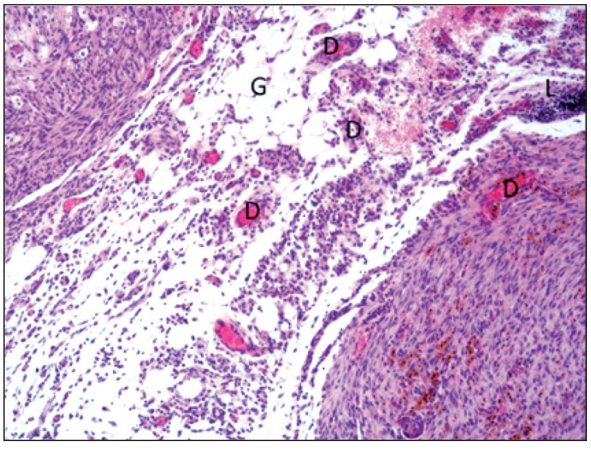

FIGURE 7: Experiment group. Nerve section. **L:** Lymphocyte infiltration areas (H&E ×100).

of fat graft is also cheaper compared to adipose tissue delivered stem cells. It can be harvested from various regions of the body easily. We observed that wrapping the fat graft around the nerve coaptation zone had a positive effect on early neural regeneration in rat model.

The main drawback of our study was to analyze only the early period of neural regeneration. Further studies aim to investigate the cell migration patterns of the regenerative period would help us to understand the effect of fat grafts on neural regeneration more decent.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Burak Ersen, Ramazan Kahveci;* ***Design:*** *Burak Ersen, Ramazan Kahveci;* ***Control/Supervision:*** *Burak Ersen, Ramazan Kahveci;* ***Data Collection and/or Processing:*** *Burak Ersen, Nesrin Uğraş, Gökhan Ocakoğlu;* ***Analysis and/or Interpretation:*** *Burak Ersen, Ramazan Kahveci;* ***Literature Review:*** *Burak Ersen;* ***Writing the Article:*** *Burak Ersen; Critical Investigation: Ramazan Kahveci;* ***References and Fundings:*** *Burak Ersen, Nesrin Uğraş;* ***Materials:*** *Burak Ersen.*

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