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Research Article

Morphological and Functional Impact of Different Techniques on Limb Dissection: An Experimental Study in a Rat Model

Shayan Abdollah Zadegan,[1,2] Masoumeh Firouzi,[1,2] Mohammad Hossein Nabian,[1,3,*] Leila Oryadi Zanjani,[1,3] Mohsen Nategh,[1,2] and Reza Shahryar Kamrani[3,4]

[1]Research Center for Neural Repair (RCNR), Animal Laboratory Department, University of Tehran, Tehran, IR Iran
[2]Tissue Repair Lab, Institute of Biochemistry and Biophysics (IBB), University of Tehran, Tehran IR Iran
[3]Department of Orthopedic and Trauma Surgery, Shariati Hospital, Tehran University of Medical Sciences, Tehran, IR Iran
[4]Joint Reconstruction Research Center, Tehran University of Medical Sciences, Tehran, IR Iran

**Corresponding author*: Mohammad Hossein Nabian, Department of Orthopedic and Trauma Surgery, Shariati Hospital, Tehran University of Medical Sciences, P. O. Box: 1411713135, Tehran, IR Iran. Tel: +98-9126305095, E-mail: dr.nabian@gmail.com

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Background: Comparative studies on the effect of different tissue dissection techniques concerning adhesion, scar formation and functional impact on nerve and muscles are scarce, especially in orthopedic traumatic conditions.
Objectives: In the present experimental study, we aimed to investigate the impact of the choice of dissection technique on the peripheral nerve and surrounding tissues in a rat model of lower limb injury.
Materials and Methods: Forty adult female Wistar rats were divided into five groups (n = 8): In group 1, surgical blade was used to simulate sharp dissection; in group 2, a standard surgical forceps was used to simulate dissection with muscle contusion; In group 3, a small piece of compressed steel wool was used to simulate abrading during dissection; In group 4, extracorporeally minced muscles were used to simulate aggressive dissection; In group 5, a bipolar electrocoagulation instrument was used to simulate electrocautery dissection. During 3 weeks, the five groups were evaluated with functional assessment by toe out angle (TOA), morphological assessments by Petersen gross anatomical scale and histological assessment by scar formation index.
Results: The mean TOA was higher significantly in group 5 ($P < 0.001$). The largest mass of gross scar and the highest nerve adherence (grade 3) was detected in group 4 ($P = 0.002$). Scar index in all experimental groups was significantly higher than normal ($P < 0.001$). Group 4 showed the highest scar index ($P = 0.000$) and group 1 showed the lowest scar index.
Conclusions: Deep dissection with sharp cutting tools, results in low scar formation, tissue adhesion and subsequent indirect nerve injury. Electrocoagulation dissection may have side effects on adjacent nerves. Aggressive dissection, abrading during dissection and contusion should be avoided.

Keywords: Dissection; Tissue Adhesions; Wound Healing; Electrocoagulation; Peripheral Nerves

1. Background

In the past decade, numerous studies were performed to compare the safety and efficacy of electrocautery versus scalpel for tissue dissection (1, 2). Although common practice by most surgeons is to make skin incisions with a scalpel and to dissect the deeper tissues with electrocoagulation, the best choice remained controversial (2).

Most studies assessing the effects of dissection techniques on the outcome of surgery are in the field of abdominoplasty and mastectomy. Such studies are designed to examine the effect of surgical technique mainly on the skin incision and occasionally on deep dissection (1, 2). Using scalpel versus diathermy (electrocautery) was frequently compared in these studies because diathermy rises concerns about collateral heat damage and impaired wound healing based on experimental studies (2-5). However, clinical data do not support the experimental results (1, 2). In other fields of surgery like cardiovascular surgery, using electrocautery for dissection and harvesting is still controversial and a combined approach is recommended (6, 7).

To the best of our knowledge, there is limited comparative data on the effect of different tissue dissection techniques concerning adhesion, scar formation and functional impact, especially in orthopedic traumatic conditions. There are a few studies on the impact of dissection method on peripheral nerves, which are mainly based on nerve sparing resection techniques for patients with cancer (8).

2. Objectives

In the present experimental study, we aimed to investigate the impact of the choice of dissection technique on functional, morphological and histological changes of the peripheral nerve and surrounding tissues in a rat lower limb injury model.

3. Materials and Methods

3.1. Study Design

Forty adult female Wistar rats (180 - 200 gr) were randomly assigned into five experimental groups (8 in each group). The rats were kept four per cage in a temperature-controlled room with 12 - 12 hours light/dark cycles and ad libitum access to food and water. One week before surgery, animals were housed for adaptation and training. The animals of five groups were evaluated with functional, morphological and histological assessments during three weeks of experiments. Assessments were conducted by an observer blinded to the interventions. All experiments were approved by the ethical committee of Tehran university of medical sciences.

3.2. Surgical Procedures

The rats were anesthetized with an intraperitoneal injection of Ketamine (100 mg/kg) and Xylazine (5 mg/kg). Under aseptic conditions and using standard gluteal muscle-splitting approach, the right sciatic nerve was exposed and isolated from surrounding muscle bed. While the nerve was protected, five different models of dissection were performed over the length of 1.5-mm of the exposed surface of the adductor and biceps femoris muscles.

In group 1 (model of sharp dissection with scalpel), surgical blade number 15 was used to make five axial and three vertical slashes, each 2 mm-deep (9). In group 2 (model simulating dissection with muscle contusion), the muscles were compressed with a standard surgical forceps at maximum closure point for 10 seconds (10). In group 3 (model simulating instrumental dissection/abrading during dissection), 20 scratches were made on the muscles surface with a small piece of compressed steel wool (11). In group 4 (model simulating aggressive dissection), adductor muscle and a 5-mm strip of the anterior border of the biceps femoris were removed and minced extracorporeally in a Petri dish. Then, the muscle pieces were returned to the periphery of the nerve (12). In group 5 (model of electrocautery dissection), the surface of the muscles was cauterized in six different areas using a bipolar electrocoagulation instrument (Micro-3 Plus; MIZUHO, Tokyo, Japan) (13).

Finally, muscles and fascia were closed with simple 4/0 vicryl sutures and the skin was sutured in continues fashion using 4/0 nylon suture. Picric acid (2, 4, 6-trinitrophenol, saturated aqueous solution, Sigma-Aldrich Co. St. Louis, MO) was used for autotomy prevention (14).

3.3. Functional Assessment

To assess sciatic nerve function, the TOA (Toe out angle) was measured weekly. TOA was previously described by Varejao and colleagues as an indicator of foot rotation during the normal walk (15). It was defined as the angle between direction of walking and a reference line in the foot (the line from the calcaneus to the tip of the third digit) during mid-stance phase of gait.

The test was performed in a transparent walking track with 1 meter length and 15 cm width. A mirror was placed beneath the pathway with 45° angle to reflect the view of the inferior surface of the rat hindlimb. A digital high-speed camera (Sony, HDR-SR12 high definition, Japan) was used to record the walking process. Mid-stance frames of the right foot were analyzed for TOA calculation.

3.4. Morphological and Histological Assessments

At the end of study, all animals were killed for evaluation of gross morphology and histology. In each group (n = 8), four animals were assigned for gross morphological evaluation and the other four used for histological assessments.

In gross morphological evaluation, skin closure, muscle fascia closure and nerve adherence to the surrounding muscle were determined by gross assessment with the numerical scheme described by Petersen et al. (Table 1) (11).

Table 1. Numerical Scheme for Macroscopic Evaluation [a]

Tissue	Definition
Skin and Muscle Fascia	
Grade 1	Skin or muscle fascia entirely closed
Grade 2	Skin or muscle fascia partially open
Grade 3	Skin or muscle fascia completely open
Nerve Adherence/Separability	
Grade 1	No dissection or only mild blunt dissection
Grade 2	Some vigorous blunt dissection
Grade 3	Sharp dissection required

[a] Originally described by Petersen et al. (11).

For histological assessment, en bloc specimens of the sciatic nerve and peripheral tissues were removed, fixed in 4% paraformaldehyde and embedded in paraffin for microscopic assessments. Serial sections of 6 μm were stained with Masson trichrome for connective tissue. Digital photos of stained sections were used to calculate scar index using image-J software (16). Scar index was calculated by dividing the cross-sectional area of the scar tissue by the whole area of the nerve (epineurium and the nerve) (17).

3.5. Statistical Analysis

Data was analyzed by SPSS 19 software (Armonk, NY: IBM Corp) using analysis of variance (ANOVA) or non-parametric tests. P value < 0.05 was considered statistically significant.

4. Results

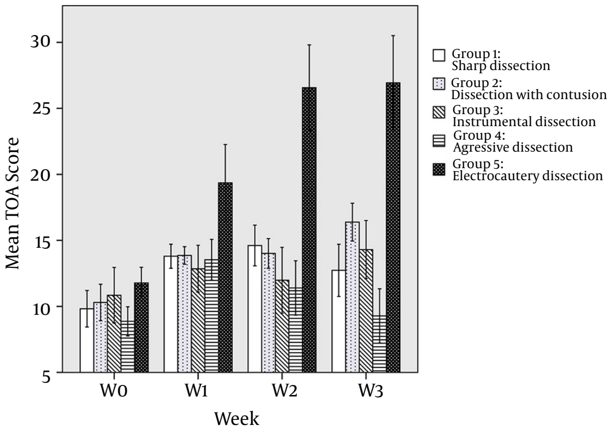
4.1. Functional Assessment

Differences among baseline TOA scores at week 0 were insignificant. The time course of TOA was significantly different in group 5 (P < 0.001, Repeated measure ANOVA, Bonferroni post-hoc test) and there was no significant difference between other experimental groups. The mean TOA in group 5 (Electrocautery) was higher compared with other groups, demonstrating a higher degree of foot rotation and impaired functional outcome (Figure 1).

4.2. Morphological Assessment

At the end of study, sciatic nerves were re-exposed and evaluated using the Petersen numerical grading scheme (11). No sign of infection was detected. The largest mass of gross scar and the highest nerve adherence (grade 3 in Petersen scale) was detected in group 4 (P = 0.002, Kruskal-Wallis test). Detailed scores are shown in Table 2.

Figure 1. Mean TOA (± SE), at 0, 1, 2, and 3-Week Time Points



The mean TOA score was significantly different in group 5 (P < 0.001).

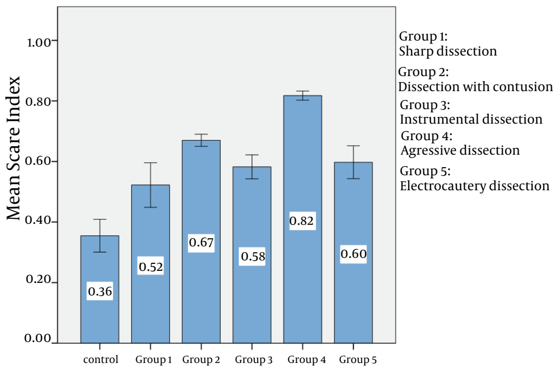
Table 2. Gross Morphology of Skin, Muscle, and Nerve Using the Petersen Grading Scale

Group Number	Model of Dissection	Rat Number	Skin and Muscle Fascia	Nerve Adherence
1	Sharp dissection/Scalpel	1	1	1
		2	1	1
		3	1	2
		4	1	1
2	Dissection with muscle contusion	5	1	2
		6	1	2
		7	1	1
		8	1	1
3	Instrumental dissection	9	1	3
		10	1	3
		11	1	2
		12	1	2
4	Aggressive dissection	13	1	3
		14	1	3
		15	1	3
		16	1	3
5	Electrocautery dissection	17	1	2
		18	1	1
		19	1	1
		20	1	2

4.3. Histological Assessment

Scar index was calculated on Masson trichrome-stained slides. In comparison of scar indexes, non-experimental leg (left leg) served as the control group. Scar index in all experimental groups was significantly higher than the normal group ($P < 0.001$, One-way ANOVA, Bonferroni post-hoc test, Figure 2). The highest mean scar index (0.82 ± 0.01) was calculated in group 4 (aggressive dissection) and was significantly higher than all the other groups ($P = 0.000$ group 1, 3, 5; $P = 0.015$ group 2, One-way ANOVA, Bonferroni post-hoc test, Figure 2).

Figure 2. Mean Scar Index (± SD) Calculated on Masson Trichrome-Stained Slides



Scar index was significantly higher in group 4 ($P < 0.001$).

The lowest mean scar index (0.52 ± 0.07) was seen in group 1 (sharp dissection with scalpel), which was significantly lower than group 2 ($P = 0.015$) and group 4 ($P = 0.000$). There was no significant difference between scar indices in groups 1, 3 and 5 ($P > 0.05$, Figure 2).

5. Discussion

The present study investigated the impact of different dissection techniques on tissue adhesion, scar formation and function of rat sciatic nerve. We found that all dissection methods could produce scar tissue to some degree, even dissection with sharp cut or instrumental dissection. As expected, aggressive dissection produced the highest amount of scar tissue, which was possibly due to necrotic tissue (18, 19). We found that sharp dissection had the minimal adhesion, scar formation and nerve injury and the electrocautery had the most nerve injury, despite acceptable scar formation and surrounding tissue adhesion.

The adverse effect of electrocautery on the nerve function was higher than other groups, even with the same amount of scar tissue. Previous experimental studies raised concerns about collateral heat damage and impaired wound healing after diathermy (2-5). In an experimental study, Rappaport and colleagues found that cutting with coagulation current significantly reduced midline wound tensile strength in fascia and increased tissue necrosis, inflammation and adhesion, compared with the scalpel or cutting current. (4) Similarly, Ozgun and colleagues reported that diathermy causes more inflammation, tissue injury and necrosis in abdominal fascia compared to scalpel (5, 20). Rubino and colleagues reported the same disadvantage of electrosurgery in muscles of rabbit (21). They found that using harmonic (ultrasonically activated) scalpel for dissection of skeletal muscle may reduce acute inflammation compared with the monopolar electrosurgical scalpel (21). In spite of these studies, there is a body of clinical evidence that supports the safety of diathermy and rejects the possibility of excessive inflammation (1, 2). However, there is still some controversies (20, 22). Most these studies performed on the effect of diathermy on skin incision, not deep dissection (23).

Kauff and colleagues in a study on pigs assessed the quality of pelvic autonomic nerve preservation after different dissection techniques (8). They evaluated four different techniques (scissors, ultracision, monopolar diathermy and waterjet) for low anterior rectal resection (LARR) (8). In their study, pelvic autonomic nerve preservation was evaluated with electromyography of the internal anal sphincter. It was found that scissors, ultracision and monopolar diathermy might have comparable nerve-sparing potentials (8). They used coagulation mode for diathermy. In contrast, we found some adverse effects for electrocoagulation of the peripheral tissue of the sciatic nerve.

To sum up, diathermy has the advantage of shorter operative time and a bloodless dissection (20, 24). On the other hand, sharp cut with scalpel gives a more precise surgical dissection and reduce the risk of thermal injury and inflammatory response (20, 23, 24).

In conclusion, deep dissection with sharp cutting tools results in low scar formation, tissue adhesion and subsequent indirect nerve injury; however, care must be taken to avoid direct nerve injury. Marginal necrosis in the plan of dissection should be removed at the end of procedure, because necrotic tissue could induce massive scar formation. There is experimental evidence to raise concerns about possible side effects of electrocoagulation dissection, especially on adjacent nerves; further experimental and clinical studies are needed. Aggressive dissection, abrading during dissection and contusion should be avoided.

Authors' Contributions

Shayan Abdollah Zadegan: conceiving and design the study, writing the manuscript. Masoumeh Firouzi: study design and conceiving, critical revisions. Mohammad Hossein Nabian: analysing and interpreting the data, writing manuscript, final approval. Mohsen Nategh: conceiving study, analysing study. Leila Oryadi Zanjani: conceiving study, critical review. Reza Shahryar Kamrani: study design, critical review.

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