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Immediate *versus* delayed primary nerve repair in the rabbit sciatic nerve

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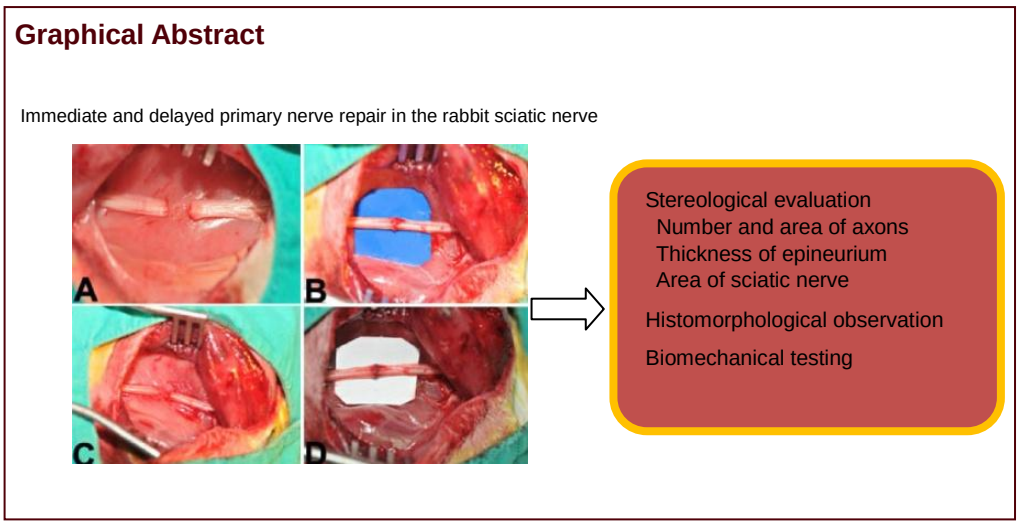
Graphical Abstract



Abstract

It is well known that peripheral nerve injury should be treated immediately in the clinic, but in some instances, repair can be delayed. This study investigated the effects of immediate *versus* delayed (3 days after injury) neurorrhaphy on repair of transected sciatic nerve in New Zealand rabbits using stereological, histomorphological and biomechanical methods. At 8 weeks after immediate and delayed neurorrhaphy, axon number and area in the sciatic nerve, myelin sheath and epineurium thickness, Schwann cell morphology, and the mechanical property of nerve fibers did not differ obviously. These results indicate that delayed neurorrhaphy do not produce any deleterious effect on sciatic nerve repair.

Key Words

neural regeneration; delayed peripheral nerve repair; sciatic nerve; peripheral nerve injury; stereology; microscopy; biomechanics; neuroregeneration

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Conflicts of interest: None declared.

INTRODUCTION

Peripheral nerve injuries affect all age groups and can have a devastating impact on patient's job and daily activities[1]. Peripheral nerve injuries frequently occur as a result of accidental trauma, deliberate surgery, or acute compression[2]. These injuries are often the result of traumatic events, such as an open fracture or wound, but they can also present latently after a peripheral nerve block or while observing a compartment syndrome. Generally, peripheral nerve injuries should be treated immediately in the clinic, but in some instances, repair can be delayed[1].

The cut and repaired nerve ends are subjected to various mechanical forces that must be recognized and managed appropriately to preserve blood flow and allow nerve healing. Despite multiple treatment options are available (immediate or delayed repair, nerve grafting, nerve transfer, nerve transplant, nerve conduits), biomechanical requirements are the same for any type of repair[3].

An understanding of subsequent cellular changes after nerve injury is essential for determining the proper timing and technique of nerve repair to produce optimal functional results[4]. It has been reported that delayed repair (pre-degeneration) may benefit neural regeneration. Since the proliferation of Schwann cells starts 24 hours after peripheral nerve injury and accelerates 3 days after injury[5]. To the best of our knowledge, there have been no studies regarding immediate and delayed sciatic nerve repair using biomechanical and stereological methods. In this study, we investigated the effects of immediate *versus* delayed neurorrhaphy on sciatic nerve repair using biomechanical testing and histomorphological assessment.

RESULTS

Quantitative analysis of experimental animals

Twenty-eight rabbits were randomly and evenly divided into two groups: immediate primary nerve repair (INR) group and delayed primary nerve repair (DNR) group. Rats in the INR and DNR groups received immediate and delayed (3 days after injury) neurorraphy respectively after sciatic nerve injury.

Stereological results

Stereological analysis showed that there were no significant differences in axon number and cross-section area, epineurium thickness, and peripheral nerve cross-section area between INR and DNR groups ($P > 0.05$; Figure 1).

Histomorphological results

Through light microscopy, myelinated nerve fibers exhibited normal structure and Schwann cells appeared normal, and the myelin sheaths were intact in the INR group (Figure 2A, C). Also in the DRN group, the majority of myelinated axons were intact and Schwann cells appeared normal (Figure 2 B, D)

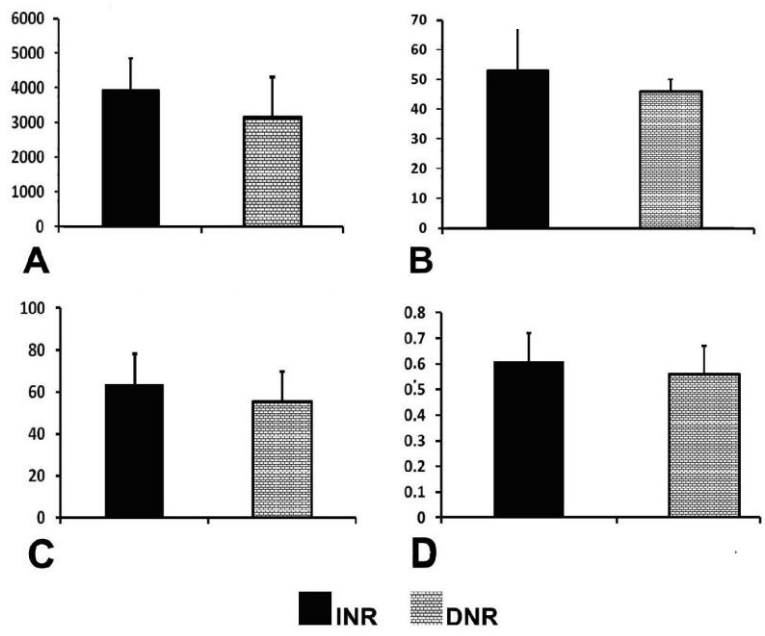

Figure 1 Axon number (A), axon cross-section area (μm^2; B), epineurium thickness (μm; C) and peripheral nerve cross-section area (mm^2; D) in the immediate primary nerve repair (INR) and delayed primary nerve repair (DNR) groups.

All measurement data are expressed as mean ± SEM. Nonparametric Mann-Whitney *U* test was used for difference comparison between groups.

Biomechanical results

The tensile strength of nerve fibers was similar between INR and DNR groups, and there was no significant difference in tensile strength of nerve fibers between INR and DNR groups ($P > 0.05$; Figure 3).

DISCUSSION

Repair of a peripheral nerve is expected to be able to resist internal and external tensions during the period of time needed for injury healing, a primordial step permitting the occurrence of axonal growth followed by functional recovery. Injuries to the peripheral nerve occur more frequently in regions such as the wrist, hand and fingers, all structures with wide amplitude of movement. These injuries are frequently associated with fractures and injuries to nearby tendons, with repair of all injured structures, including fixation of fractures and tendon repair, being always performed in a single surgical intervention.

Primary nerve repair has been preferred since 1950 and succeeded by microsurgical methods[6]. The current "gold standard" for the treatment of peripheral nerve injury is immediate epineurial repair with nylon suture[7]. We preferred epineurial repair method to fascicular method for simple and common. Proponents argue that group fascicular repair is better because axonal realignment is more accurate with this technique. However, others have shown that there is no functional difference in outcome between epineurial and group fascicular repair[8].

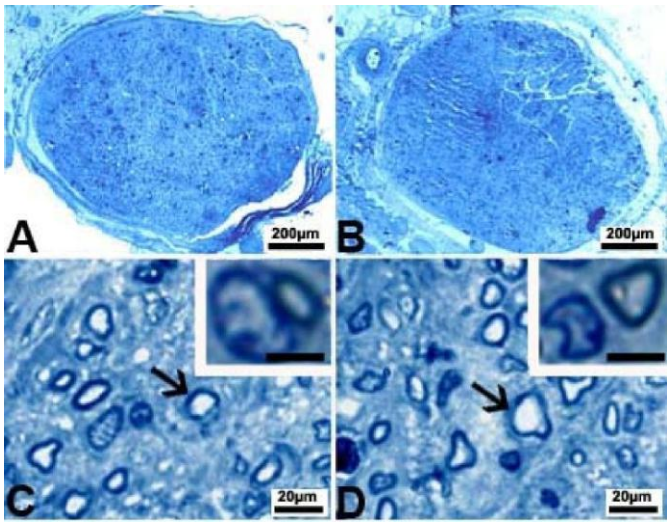

Figure 2 Light microscopic images of transected sciatic nerve after toluidine blue staining in the immediate primary nerve repair (INR; A, C) and delayed primary nerve repair (DNR; B, D) groups.

(A) and (B) are lower magnified views of the INR and DNR groups, respectively. Black arrows indicate normal axons and insets show normal Schwann cells and axon in (C) and (D). Scales are 10 μm in insets.

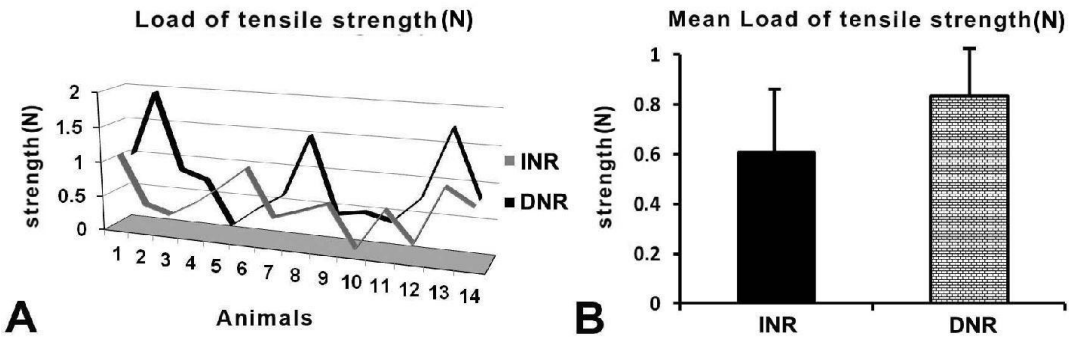

Figure 3 Tensile strength of sciatic nerve in the immediate primary nerve repair (INR) group and delayed primary nerve repair (DNR) group.

(A) Tensile strength of rabbits in the INR and DNR groups. Analysis of variance and *post-hoc* tests (Fisher's protected least square difference) were used for difference comparison between groups. (B) Mean values of tensile strength of sciatic nerve in the INR and DNR groups. All measurement data are expressed as mean ± SEM. Nonparametric Mann-Whitney *U* test was used for difference comparison between groups.

Traditionally, the results of nerve repair have been considered depending on patient/injury factors and technical factor. Injury factors include the age of the patient, mechanism of injury, and the particular nerve suffering from injury (*i.e*., pure motor *versus* mixed). Surgeons can control the results related to surgical techniques but not the injury part. Technical factors have primarily focused on fascicular misalignment and the strength of repair is considered as a cause of suboptimal results. Although there may be some benefits in doing group fascicular repairs relative to pure epineurial repairs, the anticipated benefits of doing fascicular repairs have not been demonstrated. It has been postulated that increased intra-neural scarring counteracts better fascicular alignment produced by fascicular repairs[9].

Stereology is a number of mathematical and statistical methods that allows the evaluation of three-dimensional structural information from two-dimensional sections (or slices)[10-12]. This allows researchers to derive important quantitative and structural knowledge, such as the volume, surface area or length of the biological objects and also number of cells and axons[13-15]. There are many studies on peripheral nerve repair[16-17]. But only few studies compared immediate and delayed peripheral nerve repair and none of these studies are based on a quantitative gold standard technique like stereology[18-19].

Results from this study showed that there were no significant differences in axon number, axon cross-section area, epineurium thickness, and peripheral nerve cross-section area between the INR and DNR groups, which were confirmed as stereological and histological findings.

Perineurium is the major load-carrying connective tissue of the nerve. For these reason, researchers increased number of sutures in suture because immediate epineurial suture can sustain nerve endings. An increased number of strands get increased repair power but nerve injury and scar formation rise by the manipulation[9, 20].

Temple *et al* [21] showed that by 8 weeks after neurorraphy, the repaired nerve had achieved only 63% strength of the control group. Gapping significantly leads to lower forces. Thus, for a period of time, the nerve repair depends on the sutures to prevent disruption and gapping[9]. The effect of various gauge suture material was not a variable in this experiment. We selected 9-0 nylon based on the study by Giddings et al in which 8-0, 9-0 and 10-0 nylon sutures were studied in cadaveric median nerves[22]. They concluded that 10-0 was too weak, as the sutures snapped before pulling out, with 9-0 nylon providing the highest strength of repair with the finest material.

In the study, the biomechanical properties of intact nerves have been studied in rabbit sciatic nerves. We performed epineurial suture 1 mm purchase length bite. So, at the end of healing period of time (6 weeks), there was no significant difference in biomechanical test results between INR and DNR groups. Goldberg *et al* [23] did not find a difference in repair strength or stiffness between one and two mm of purchase bite. Baoguo *et al* [24] indicated that the connective tissue of the epineurium forms a layer of fiber membrane at the third day. Because of this, we did not increase the number of sutures of nerve endings to strengthen epineurial repair but wait until epineurial tissue thickened with edema and fibrosis. We did circular sutures of epineurial sheath with stitches placed at equal distances. This facilitates manipulation and stronger suture of epineurial tissue. We only evaluated the results of one group (*i.e*., 3 days after injury) of delayed nerve repair and we did not perform electrophysiological tests postoperatively. These are the limitations of this study.

Taken together, there were no significant differences in biomechanical, stereological and histomorphological findings between the immediate and delayed nerve repair, and delayed nerve repair did not lead to any deleterious effect on healing of injured nerve.

MATERIALS AND METHODS

Materials

A total of 28 male New Zealand rabbits, weighing 2–2.5 kg, were obtained from Surgery Research and Application Center, Ondokuz Mayıs University, Turkey and included in this study. This study was approved by Animal Ethics Committee, Ondokuz Mayıs University, Turkey. Two groups of rabbits were housed in separate cages and raised under conventional laboratory conditions before experimentation.

Methods

Neurorrhaphy

Two groups of rabbits were anesthetized by intraperitoneal injection of Ketamine® (Ketasol 90 mg/kg; Richter Pharma AG, Wels, Australia), Xylazine® (Rompun 10 mg/kg; Bayer, Germany) and 0.05 mg/kg atropine.

The right sciatic nerve of each rabbit was exposed

through a dorsolateral incision and by dissecting the hamstring muscles[25]. With the aid of an operating microscope, the nerve was dissected from its surrounding tissue and isolated with a plastic sheet. The nerve was transected 20 mm below the sciatic notch using micro scissors and the ends were trimmed with a razor blade; a wooden spatula served as a cutting board by using an operating microscope. In the INR group, the end-to-end neurorrhaphy was completed with four equidistant epineurial sutures of 9-0 nylon. The epineurial knots were placed 1 mm from the cut to end. The thigh incisions were then closed and the rabbits recovered from subcutaneous analgesia (0.05 mg/kg buprenorphine). The same neurorrhaphy procedure was also applied to the DNR group with 3-day delay. The entire surgical procedure was summarized in Figure 4.

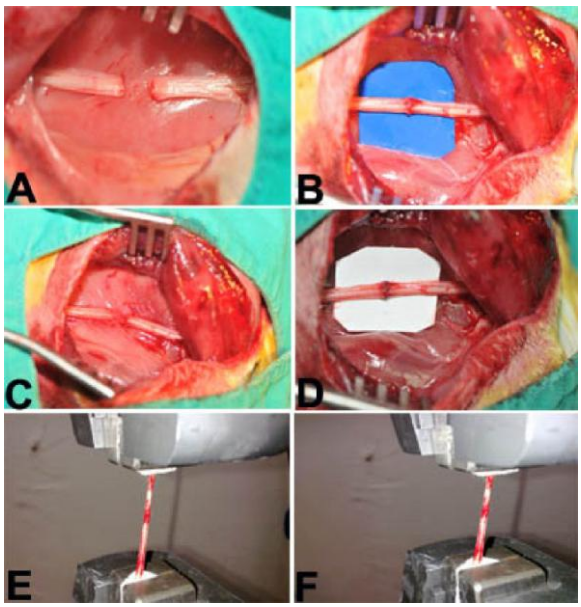

Figure 4 Surgical procedure and biomechanical tests of immediate primary nerve repair (INR) and delayed primary nerve repair (DNR) groups.

(A) Transected nerve. (B) Immediate nerve repair. (C) Transected nerve after 3 days. (D) Delayed nerve repair. (E, F) Biomechanical tests of immediate and delayed nerve repair.

After surgery, all rabbits were returned to their cages and fed water and standard chow during 6 weeks. At the end of the sixth week post-operation, all rabbits were sacrificed, and sciatic nerve samples were removed for stereological, histomorphological and biomechanical analyses.

Histomorphological analysis

Transverse sections of sciatic nerves were observed under light microscope (Leica, Tokyo, Japan) after staining with toluidine blue.

Stereological analysis

A nerve segment 5 mm distal from the anatomotic site was harvested. Subsequently, the nerve segment was fixed with 5% glutaraldehyde in 0.1 mol/L phosphate buffer (pH 7.4) and processed for electron microscopic examination. Semi-thin sections of 1 μm thickness were cut by microtome and then stained with 1% toluidine blue. Myelinated axon number, axon cross-section area, and the thickness of myelin sheath and epineurium as well as peripheral nerve cross-section area were estimated using computer-assisted stereological analysis system (CAST GRID®, Computer Assisted Stereological Toolbox, version 2.00.03; CA, USA). This stereological workstation, composed of a digital camera (JVC, Tokyo, Japan) and a light microscope (Leica, Tokyo, Japan), was used for axon number[26]. A counting frame was placed on a monitor, and the sampled area was chosen by a systematic uniform random manner *via* the dial indicator controlled specimen stage[26]. This ensures that all locations within a nerve cross-section were equally represented. To obtain an estimation of total nerve fiber number in an unbiased manner from nerve cross-section, 'the unbiased counting frame' in area was utilized[27-28]. A counting frame was placed onto sections by systematic uniform random manner and appropriately sampled nerve fibers were counted[29-31]. This ensures that all locations within a nerve cross-section were equally represented and that all axon profiles are sampled with an equal probability regardless of shape, size, orientation, and location[32].

Biomechanical testing

Nerve specimens were mounted in a servo-hydraulic testing machine (Instron 8300, Norwood, MA, USA). Nerves were then loaded under displacement control at 6 mm/min. Gapping between the three mm markings was recorded on high-resolution video photography. Video data were analyzed on a computer and correlated with the data from the mechanical testing every 6 seconds until one mm of gapping was produced. Thus, load to gapping data was generated (Figure 4).

Statistical analysis

Statistical analysis was performed using SPSS 17.0 software (SPSS, Chicago, IL, USA). Analysis of variance, *post-hoc* tests (Fisher's protected least square difference) and nonparametric Mann-Whitney *U* test were used for difference comparison between groups. Mean values were considered to be significantly different at $P < 0.05$.

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