Reactive Properties of Epi- and Perineurium: Experimental and Morphological Basis for Nerve Suture Technique

V. L. Kovalenko, V. I. Shevtsov, M. M. Shchudlo, and N. A. Shchudlo

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 130, No. 8, pp. 211-215, August, 2000
Original article submitted May 3, 2000

Epi- and perineurium were examined *in vitro* and during regeneration after mechanical damage. Epithelial type of culture growth and reparative regeneration of the perineurium were established, connective tissue of the epineurium served as the substrate and trophic factor in these processes. We used histogenetic method of coaptation and of epi- and perineural micosutures in a damaged nerve trunk.

Key words: *regeneration; nerve; nerve sheats; tissue culture*

Histogenetic type of perineural cells (PC) is now extensively discussed [7]. Attempts to refer these cells to fibroblasts [8] disagree with immunohistochemical data [6]. There are contradictory data on the regenerative potential of the perineurium [9,11,12]. Reactive properties of the epi- and perineurium as the objects of surgery are of particular importance in light of extensive use of microsurgical technique for reconstruction of nerve trunks [1,3].

To elucidate the histogenetic nature of the epi- and perineurium and to provide the technical and methodological bases for suturing damaged nerve trunks we examined them in culture and during reparative regeneration after mechanical damage.

MATERIALS AND METHODS

Epi- and perineurium explants (n=134) from rabbit sciatic nerve were cultured on coverslips without substrate in medium 199 supplemented with 10% fetal calf serum and chick embryo extract at 37°C for 1-30 days. The explants were impregnated with $AgNO_3$ according to Ranvier, stained with iron hematoxylin, and activity of nonspecific phosphomonoesterases was determined according to Gomori. Reparative regeneration was studied on 40 male rabbits aged 1.5-2 years. Sciatic nerve in the middle third of the femur was prepared and the epi- and perineurium were dissected longitudinally with Graefe's knife. In series I (n=6), the length of dissection was 1 cm. In series II (n=34), a special sharp and curved spatula was inserted through this dissection (3 mm) between the perineurium and nerve fibers in the proximal and distal directions. The perineurium was scarified with the spatula without penetrating the connective tissue of the epineurium. The nerve trunk was submerged into the wound (no sutures were put on the nerve) and the muscles and skin were sutured with catgut and silk, respectively. The animals were sacrificed 3 h-70 days after surgery. For histological examination, the operated nerves were cut into two parts (Fig. 1). One fragment was fixed in 10% formaldehyde, embedded in paraffin, and slices were stained routinely. In the second fragment, the perineurium was dissected under a stereomicroscope contralaterally to surgical dissection and flat preparations of the nerve sheaths were prepared, stained or impregnated.

Regeneration of transected sutured sciatic nerve was studied in 45 experiments: in 32 adult mongrel dogs (experiment) a specially developed epi-perineural microsuture was applied after coaptation of the nerve ends by relaxation suture between the major

South Ural Research Center, Russian Academy of Medical Science.
Address for correspondence: gip@rncvto.kurgan.ru. Shchudlo M. M.

0007-4888/00/0008-793$25.00

nerve bundles. In control animals (n=13) epineurial microsurgical suture was applied as described elsewhere [10]. Both methods were applied in 3 experimental series: primary suture after neurotomy (series I), suture after compensation of segmentary nerve defect (15-33% femur length) by changing joint position (series II) or temporary shortening of the femur followed by distractional osteosynthesis (series III).

Electrophysiological properties of the neuromuscular system in operated and contralateral limbs were examined during the experiment. The animals were killed by intravenous injection of lethal barbiturate dose at various terms of the experiment. The material for histological examination was fixed by the Haidenhein method and embedded in paraffin or celloidin. Nerve fibers were examined after fixation in 12% neutral formaldehyde in frozen sections impregnated by the methods of Bilshovsky—Gross, Campos or Rasskazova. Active regeneration of sutured nerve was determined by clinical, morphological and electrophysiological signs: 2 weeks — longitudinally arranged regenerating axons at the level of ligature canals and in the terminal part of the distal segment; 1 month — regenerating axons in the femoral nerve; 2 months — pronounced M-responses in the *gastrocnemius* and *m.tibialis anterior*; 3-4 months — disappearance of trophic disturbances, recovery of the supportive function of the limb, and appearance of regenerating nerve fibers, plexuses, and endings in the crus muscles; 6 months — the absence of weak or pathological support after physical load (running), disappearance of polyphasic M-responses, the amplitude of M-responses attained the level recorded in the contralateral extremity; 9-12 months — disappearance of asymmetry of the electromyogram, the absence of massive secondary axonal degeneration in the distal parts of the sciatic and tibial nerves. Nerve regeneration was estimated by the above listed parameters. The interval randomization criterion was used for comparison between different small samples [5].

RESULTS

After 3 days in culture, single fibroblasts (FB) migrated from the epi-perineural explants. They had round or oval nuclei surrounded by extensive cytoplasm; perinuclear zone (endoplasm) was enriched with basophilic granules and fibrils. Faintly stained homogenous peripheral cytoplasm (ectoplasm) formed processes, which contacted processes of other FB. The cells were dispersely arranged without definite orientation. After 5-7 days in culture typical FB outgrowth was observed (Fig. 2, *a*). Spindle-shaped FB adjacent to the explant were arranged perpendicular to its edges (Fig. 2, b). Distant FB are spread on the coverslip and dramatically enlarged due to wide processes. Their cytoplasm contained intensively stained granular and fibrillary endoplasm (Fig. 2, *c*) characterized by high activity of acid phosphatase (Fig. 2, *d*) and homogenous ectoplasm. FB never formed continuous monolayer, even in areas of their dense accumulation no borders between these cells were impregnated with $AgNO_3$. Generally, FB occupied significant areas on the coverslips, though mitotic figures were rarely seen.

In cultures with pronounced FB growth, the propagation of PC starts after 5-7 days of culturing. They form bundles covering the epineural connective tissue. In some regions a continuous layer of PC migrates from the explant surface onto FB outgrowth zone. PC borders are intensively impregnated with $AgNO_3$ and stained with iron hematoxylin; the cells had polygonal shape with round edges (Fig. 3, *a*). Acid phosphatase activity was revealed along PC membranes. After 7-10 days of culturing PC layer overlaps the FB outgrowth zone and spread further on the coverslip (Fig. 3, *b, c*). PC exhibit high proliferative activity and closely contact with adjacent cells. On later stages of culturing the PC monolayer becomes less dense and form cell bundles merging again into a monolayer. Giant multinuclear cells occurred on the border of the perineurium monolayer.

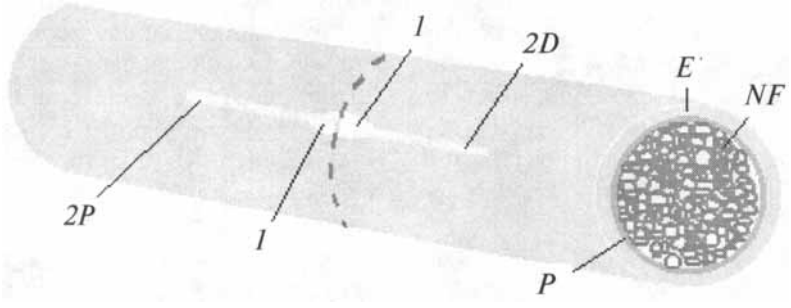

Fig. 1. Damage to nerve tunics and dissection of material for histological examination. *E*: epineurium, *P*: perineurium, *NF*: nerve fibers; *1*) penetrating lesion of epi- and perineurium; *2P* and *2D*: proximal and distal zones of isolated perineurium damage. Dotted line shows nerve section for taking specimens.

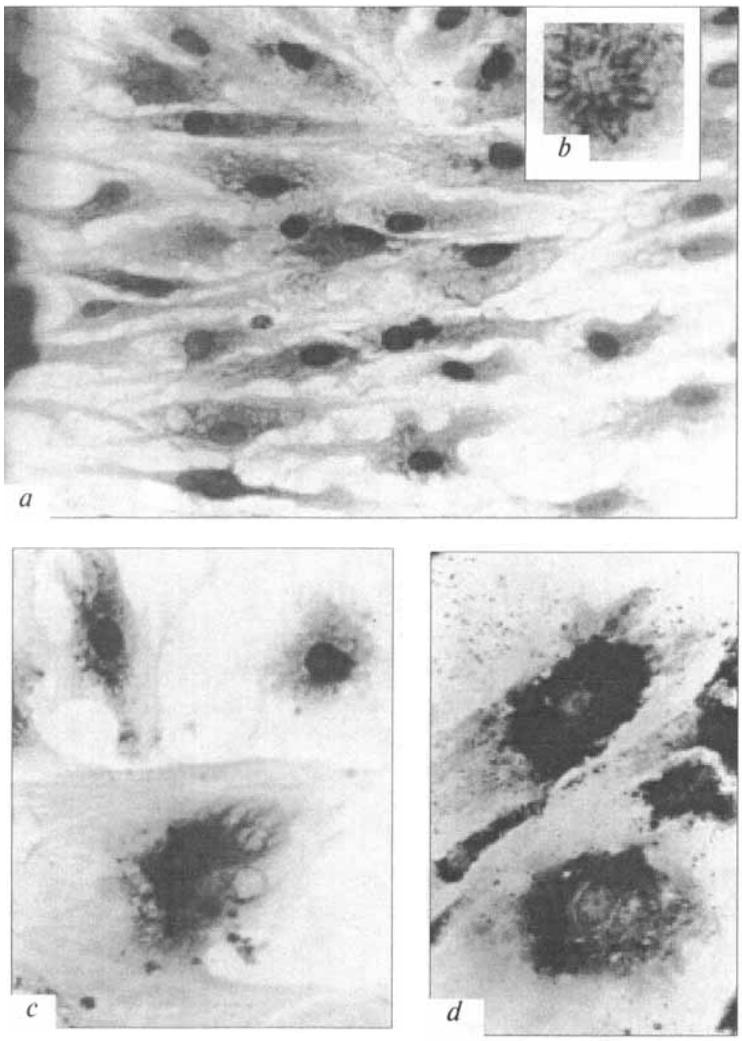

Fig. 2. Fibroblast outgrowth zone. Iron hematoxylin staining (*a-c*) and Gomori staining for acid phosphatase (*d*). *a*) the region near the epi-perineural explant, ×400; *b*) mitosis in a fibroblast, ×1350; *c*) 2 weeks and *d*) 1 month in culture, ×900.

Thus, at all stages of culturing PC retain typical features allowing to distinguish them from FB. Culturing of PC on coverslips without substrate was possible only in the presence of the epineural connective tissue forming the FB outgrowth zone and conditioning the culture medium. Thereafter, PC formed a continuous monolayer over the FB outgrowth zone. Thus, the epi-perineural interrelations typical of native nerve trunk are preserved *in vitro*. We never observed transformation of FB into PC and vise verse in primary culture.

Study of the epi- and perineural reaction showed that leukocyte infiltration appeared as soon as 3 h after damage. In the sites of penetrating lesion of the nerve sheaths, the perineurium edges were turned inside out and formed a cuff due to peculiarities of its fibrous structure. Nerve fibers formed a fungiform structure. Traumatic neuroma was presented by connective tissue scar adhered to nerve fibers. In zones of isolated perineural damage, the first signs of regeneration were noted 24 h postoperation: these were amitotic cells in the outer perineural layers distally from the lesion. By the end of day 2, the number of dividing cells increased and mitotic cells predominated. Numerous mitotic figures were observed close to wound edges (Fig. 4, *a*). Regenerating epithelium formed a layer over the wound surface (Fig. 4, *b*) and covered it by the end of day 5 postoperation. Thereafter, the number of dividing cells decreased. After 10 days, the newly formed perineurium consisted of 2-3 layers of flat and small polygonal cells. Four to five weeks after surgery, regenerating perineurium contained normal (5-6) number of cell layers.

Thus, penetrating damage to the epi- and perineurium healed via substitution regeneration with the formation of connective tissue cicatrix adjacent to nerve fibers. The perineurium was not completely restored even 70 days postoperation. In contrast, the sites with preserved epineurium were characterized by epithelial type of regeneration occurring due to proliferation of PC (primarily in its outer layers) around the wound and covering the lesion by the cell layer. This attested to trophic and supportive function of the epineurium

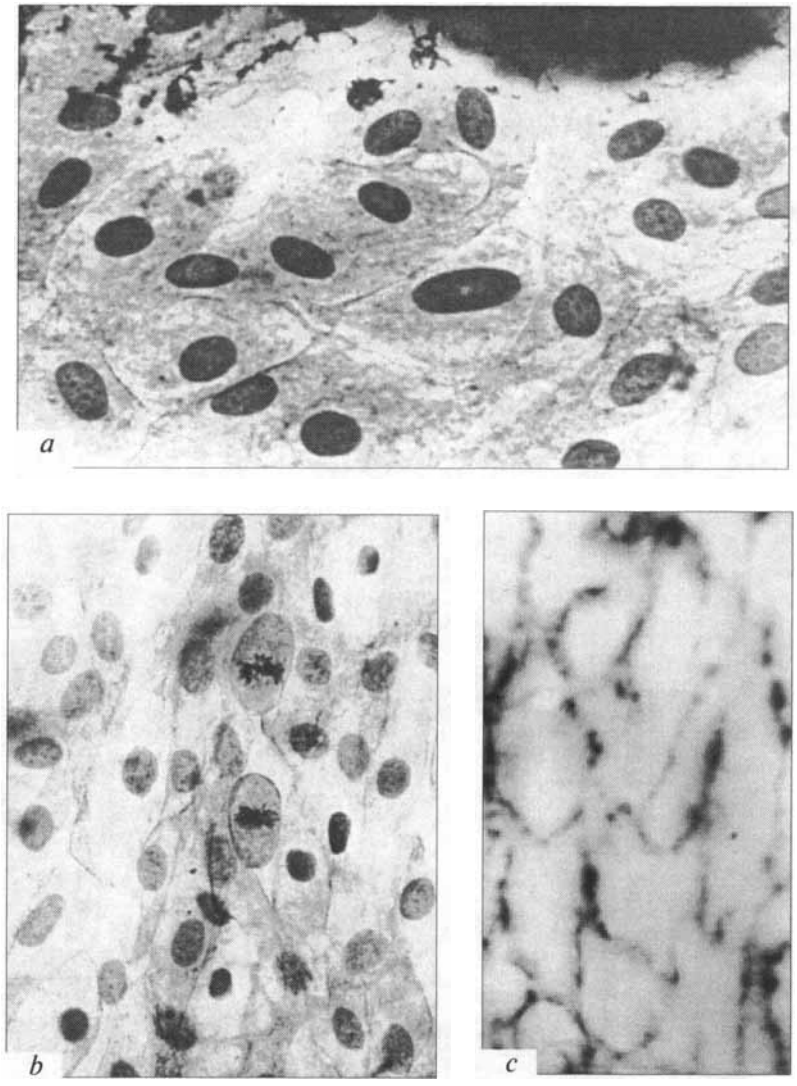

Fig. 3. Perineurium outgrowth zone near the edge (*a*) and at a distance of the explant (*b*). Iron hematoxylin staining (*a*, *b*) and Gomori staining for acid phosphatase (*c*), ×400.

possessing well developed microcirculatory system compared to perineurium containing no exchange vessels.

Thus, we elaborated a technique of nerve suture providing restitution of histogenetically different structures of the epi- and perineurium, which determines the quality of nerve regeneration and its functional recovery. The following results were observed: series I: 7 good, 2 satisfactory results (control: 2 satisfactory, 1 bad); series II: 14 good (control: 4 satisfactory, 2 bad); series III: 7 good, 2 satisfactory (control: 1 satisfactory, 3 bad); $p<0.001$ for all series.

The revealed interactions between the epi- and perineurium explain disadvantages of traditional methods of nerve sutures. Turning of the perineurium inside out interferes with regeneration of nerve fibers and is difficult to diagnose and remove during nerve suture. Epineurium suture does not allow adaptation of the perineurium edges; loose and movable epineurium allows the appearance of diastasis with a blood

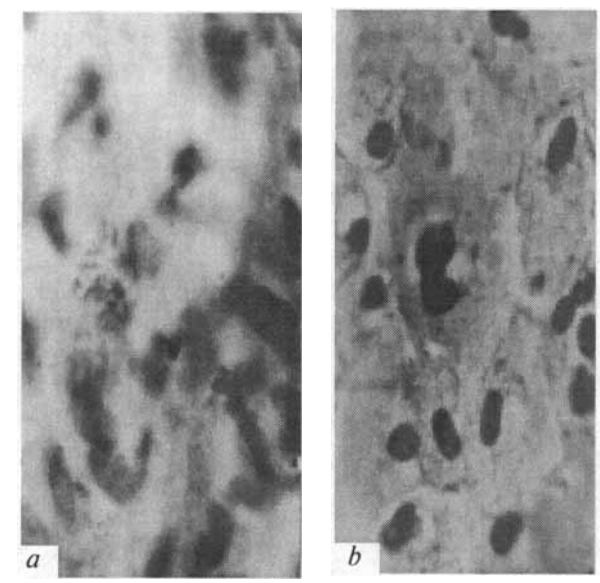

Fig. 4. Reparative regeneration of epi- and perineurium 5 days after isolated perineural lesion. Flat membrane preparations, hematoxylin staining. *a*) mitosis in a perineural cell near the wound edge, ×900; *b*) perineurocyte monolayer over the wound, ×400.

clot replaced then by cicatrix tissue, which blocks regeneration of nerve fibers. Dissection, separation, and resection of the epineurium before perineurium suture [1,4] have no effect [2], because under these conditions perineurium is deprived of its trophic and supportive basis.

REFERENCES

1. V. P. Bersnev, E. A. Davydov, and E. N. Kondakov, *Surgery of Vertebral Column, Spinal Cord and Peripheral Nerves* [in Russian], St.-Petersburg (1998), pp. 248-251.
2. I. G. Grishin, *Ann. Travm. Ortoped.*, No. 1, 23-28 (1993).
3. O. V. Dol'nitskii and Yu. O. Dol'nitskii, *Atlas of Nerve Microsurgery*, Kiev (1991).
4. *Neurotraumatology* [in Russian], Moscow (1994).
5. R. Runion, *Manual on Nonparametric Statistics. Modern Approach* [in Russian], Moscow (1982).
6. A. V. Smirnov and I. N. Sikolova, *Arkh. Patol.*, **5**, 14-20 (1995).
7. Yu. B. Chaikovskii, *Morfologiya*, **15**, No. 1, 55-67 (1999).
8. E. I. Chumasov, V. G. Seliverstova, and E. S. Kokin, *Pathological Anatomy of Surgical Diseases of the Nervous System* [in Russian], St.-Petersburg (1991), pp. 231-243.
9. S. I. Shchelkunov, *Main Principles of Cell Differentiation* [in Russian], Moscow (1977).
10. R. M. Braun, *Clin Orthop.*, **163**, No. 3, 50-56 (1983).
11. T. R. Shanthaveerappa and G. H. Bourne, *Nature*, **199**, No. 4893, 577-579 (1963).
12. T. R. Shanthaveerappa and G. H. Bourne, *Science*, **154**, No. 3755, 1464-1467 (1996).