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The Faith of Ilioinguinal Nerve After Preserving, Cutting, or Ligating It: An Experimental Study of Mesh Placement on Inguinal Floor

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Background. **Postherniorrhaphy chronic pain may be related to the trauma to the regional nerves or prosthetic mesh. This study was aimed to search the objective findings of prosthetic mesh placement on the ilioinguinal nerve in three different nerve treatment patterns with two different mesh types.**

Materials and Methods. **Thirty New Zealand rabbits were used. Bilateral ilioinguinal nerves were identified. A 2 × 1 cm standard polypropylene mesh was laid on the nerve on right side, whereas a same sized lightweight polypropylene was applied on the left after three different nerve treatments were carried out. The nerve was completely preserved in the first group [G1], cut by scissors without a further process in the second [G2], and proximal cut end was ligated with 5/0 polyglactin. Three months after the surgery, bilateral nerve samples were taken from the contiguous nerve segment for light microscopy and electron microscopy.**

Results. **Nerve protection could not prevent microscopic changes entirely. Prosthetic mesh itself seemed to cause histopathologic changes. Overall incidence of histopathologic changes in light microscopy, without taking the nerve treatment pattern into account, was somewhat lower at standard mesh side than that of lightweight mesh side. However this difference did not reach the level of significance ($P = 0.39$). When three groups were evaluated in respect to overall nerve damage without paying attention to mesh type, the highest damage rate was observed in G3 (cut and ligate). When each group was compared separately within itself for histopathologic changes, no differences were observed between heavy and light mesh sides in any group. When the microscopic changes were compared in respect to the different nerve treatment patterns on heavyweight mesh side, the rates were 12.5%, 12.5%, and 33.3%, respectively. On lightweight mesh side, all three groups exhibited similar microscopic finding rates, 37.5%, 25.0%, and 33.3%, respectively. Protection of the nerve resulted in virtually zero neuroma formation after two types of mesh use. Surgical trauma to the nerve was observed to have an obvious potential for neuroma formation. Mesh type did not affect the overall neuroma rate within the whole subject pool; both groups displayed same 40% overall neuroma development rate. The neuroma incidence was in 43.8% G2 and 72.2% in G3, however the difference did not attain level of significance ($P = 0.09$). The highest rate was observed when a lightweight mesh was used after dividing and ligating the nerve.**

Conclusions. **Light mesh could not provide a protection in subjects whose nerves were injured during surgery. Ligation of the cut end of the nerve also could not be helpful. Nerve protection still seems to be the best way for a nerve-related complaint-free postoperative period. The merit of nerve end implantation into the muscle should also be reconsidered.**

Key Words: **inguinal hernia; mesh; lightweight; heavyweight; ilioinguinal nerve; neuroma; neuropathic pain; chronic postoperative pain; experimental.**

INTRODUCTION

Prosthetic mesh repair for inguinal hernias is widely used because of its technical simplicity and low hernia recurrence rates [1]. Its complication rate is also low, but some patients complain of chronic pain in the inguinal region after surgery. This problem was first called "mesh inguinodynia" by Heise and Starling in 1998

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[2]. The incidence of chronic pain after inguinal hernia repairs has been reported to be as high as 54% [3], and an average rate of 5% is generally cited [4].

The exact etiology of post-herniorrhaphy inguinodynia is not fully understood. The sources of pain have been classified into three types: non-neuropathic, neuropathic, and nerve injuries [5]. The first one may arise from a periosteal reaction to the fixation sutures, scar tissue, or mechanical pressure of the folded mesh, whereas the other two types are directly related to the regional nerves, as indicated by their names.

The nerves of the inguinal region that surgeons encounter during hernia repair are the iliohypogastric, ilioinguinal, and genitofemoral nerves. It is possible to involve these nerves by cutting, injuring, suturing, stapling, compressing them, or by tacking them down during the operation. In addition, over time the mesh may be transformed into a mass called a "meshoma" that may impinge on the nerves [6]. Therefore, a delicate surgical approach to the inguinal floor, with correct identification of the three nerves and proper placement and fixation of a suitable mesh, are key for patient comfort during the early and late postoperative periods.

The nature of prosthetic meshes may influence regional nerves and may carry a risk for chronic pain. Lightweight meshes have been found to be advantageous in this respect. Although some controlled studies of various laparoscopic techniques and the Lichtenstein repair concluded that lightweight meshes produce more pain-free outcomes with reduced rates of chronic pain, other studies did not report better results with respect to long-term pain or foreign-body sensations [7–14].

Professor Amid of the Lichtenstein Hernia Institute has suggested that surgeons should treat regional nerves of the inguinal floor in the same manner as the laryngeal nerves of a thyroidectomy field [congress speeches, personal communication]. When a surgeon considers cutting a nerve that obstructs proper mesh placement, he or she should re-implant the cut end of the nerve after ligating it with an absorbable material [5]. However, the merits of this approach have never been specifically or objectively evaluated.

This study was designed to characterize objective findings for the ilioinguinal nerve after placement of two different prosthetic mesh types in an animal model, using three different nerve treatment patterns. The study was intended to cover all clinical scenarios for a nerve at risk of being cut during an anterior repair with mesh placement.

MATERIALS AND METHODS

Animal Subjects

The study protocol was reviewed and approved by the local ethics committee. Thirty New Zealand rabbits (average weight 2.5 kg) were used in the study. The rabbits were subjected to a 1-week preliminary conditioning period. They received standard chow and water *ad libitum* during this period. The animals were kept in a temperature- and humidity-controlled environment in separate cages.

Surgical Protocol

The protocol was based on a previously described model [15]. Preoperative prophylactic antibiotics (cefazoline 10 mg/kg) were administered intramuscularly 30 min before skin incision. The animals were anesthetized by an intramuscular injection of ketamine hydrochloride (35 mg/kg) and xylazine (5 mg/kg). The inguinal and scrotal regions were shaved and prepared with iodine solution. Bilateral inguinal areas were dissected via an inguinal incision. The ilioinguinal nerve was identified on both sides. The nerve was completely preserved in the first group [G1], cut by scissors without additional manipulation in the second [G2], and cut and ligated at the proximal cut end with 5-0 polyglactin (Surgilactin; Sutures Ltd., Wrexham, Wales, UK) in the last group [G3]. After each distinct nerve treatment was carried out, a 2×1-cm, standard monofilament, pure polypropylene mesh (Hermesh 3, >100 g/m^2, thickness 0.48 mm, small pore; Herniamesh S.r.l., Chivasso, Italy) was laid over the nerve on the right side, while a monofilament lightweight polypropylene mesh of identical size (Parietene Light, 35 g/m^2, thickness 0.40 mm, large pore; Sofradim Corp., Trevoux, France) was applied on the left. The wound was closed with 3-0 polypropylene (Atravmat, Dogsan, Turkey) in all subjects.

At 3 mo after surgery (the average duration of postoperative pain in patients), the animals were anesthetized using the same anesthetic protocol, and bilateral nerve samples were taken from the contiguous nerve segment for microscopic study. Nerve damage was considered to be present when axonal dilatation, inflammatory cells, and perineural fibrosis were observed. Neuroma formation was defined as dispersed nerve fibers and perineural fibrosis.

Light Microscopy

Nerve specimens were fixed in 10% formaldehyde, dehydrated by immersion in a series of alcohol concentrations, and embedded in paraffin. The sections were stained with hematoxylin and eosin (H and E) and Masson's trichrome and examined under a light microscope by a single histopathologist who was blinded to each sample's group assignment. Four parameters were evaluated: histopathologic changes in the nerve (axonal dilatation and degeneration, inflammatory cells, and perineural fibrosis), neuroma formation, fibrosis score (ranging from "–": no fibrosis between mesh fibers to "+++": bridge-like fibrosis between the fibers), and foreign body reaction to mesh (presence/absence of giant cells).

Electron Microscopy

Ilioinguinal nerve specimens were fixed by immersion in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 4 to 6 hours at 4°C, post-fixed in 1% osmium tetroxide for 2 h, dehydrated in an ascending alcohol series, and embedded in araldite. Semi-thin sections (1 μm) were stained with toluidine blue and observed under a light microscope. Ultrathin sections stained with uranyl acetate and lead citrate were observed under an LEO 906E Transmission electron microscope (Oberkochen, Germany). Electron microscopic evaluation was done by a single blinded histologist.

Statistical Analysis

SPSS for Windows 11.5 software was used for statistical analysis. Descriptive statistics were given as subject number or percent. The significance of the differences between the groups with regards to neural damage and neuroma formation incidences was evaluated by Pearson χ^2 test. Fisher's exact test was employed when a significant difference between the groups existed. The significance of the

difference between right and left sides with respect to mesh reaction and neural damage was evaluated using the McNemar test. A P value < 0.05 was accepted as significant.

RESULTS

One animal in G2 died on postoperative day 20; the other two groups suffered no mortality. Re-exploration of the inguinal regions in all groups after the third month showed dense fibrotic changes around the nerve over which the mesh had been placed.

Light Microscopy

Nerve protection could not entirely prevent microscopic changes. The prosthetic mesh itself seemed to cause histopathologic changes. Patchy degeneration of the axons and myelin and modest perineural thickening were observed in samples from animals with standard mesh application, even though the nerve was left intact. Lightweight mesh also caused breakdown of myelin sheaths and axonal degeneration, swelling, and dilatation. Microscopic evidence of neuroma formation was observed in some G1 and G2 subjects (Fig. 1).

Electron Microscopy

Findings in subjects with preserved nerves and standard mesh included mild perineural thickening, axonal and myelin degeneration, myelin sheath irregularity, and the appearance of vacuoles in the cytoplasm of Schwann cells (Fig. 2). Similar but milder observations were recorded when a lightweight mesh was placed over a preserved nerve (Fig. 3). Both myelinated and unmyelinated fibers were better protected in the standard mesh group.

The most prominent changes were observed in subjects whose nerves were cut, ligated, and covered with a lightweight mesh. These changes included degeneration of unmyelinated fibers, connective tissue accumulation around the endoneurium (onion bulb), extracellular matrix proliferation in the inter-axonal space, disintegration of unmyelinated axon groups, and obvious axonal degeneration (Fig. 4).

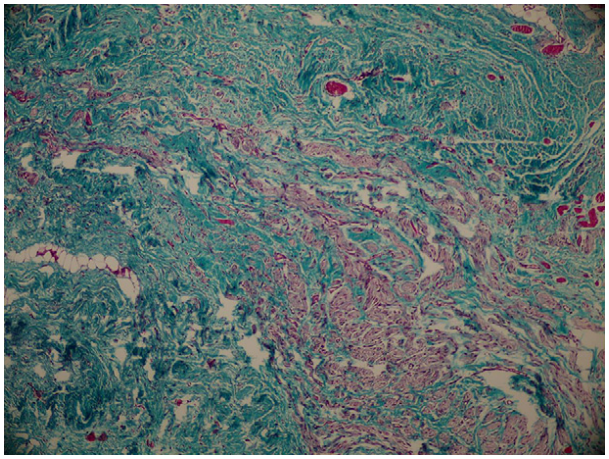

FIG. 1. Light microscopic features of a neuroma with prominent perineural fibrosis and dispersed nerve fibers following nerve division and mesh application (Masson's Trichrom; ×200). (Color version of figure is available online.)

Statistical Analysis

Without taking the nerve treatment pattern into account, the overall incidence of histopathologic changes in the ilioinguinal nerve identified by light microscopy was somewhat lower in groins treated with standard mesh compared with those treated with lightweight mesh (20% *versus* 32%). However, this difference did not reach significance ($P = 0.39$). When all three groups were evaluated for overall nerve damage (ignoring mesh type), the highest rate of nerve damage was observed in G3 (nerve cut and ligated). These rates were 37.5%, 25.0%, and 55.6%, for G1, G2, and G3, respectively ($P = 0.43$).

When animals were compared within groups for histopathologic changes, no differences were observed between the heavy and light mesh sides in any group. When the microscopic changes after each nerve treatment pattern were compared for the heavyweight mesh side, rates of nerve damage were 12.5%, 12.5%, and 33.3%, respectively. For the lightweight mesh side, all three groups exhibited similar rates of microscopic findings (37.5%, 25.0%, and 33.3%, respectively).

Protection of the nerve resulted in virtually zero neuroma formation. No neuroma formation was recorded in G1, independent of mesh type. Surgical trauma to the nerve was found to be an obvious risk factor for neuroma formation. Mesh type did not affect the overall neuroma rate within the entire subject pool; both groups displayed the same 40% overall neuroma development rate. The neuroma incidence was 43.8% in G2 and 72.2% in G3, but this difference did not reach significance ($P = 0.09$). The highest rate of nerve damage was observed when lightweight mesh was used after dividing and ligating the nerve (Table 1).

Results for the fibrosis score and giant cell appearance was also used to compare the two different meshes, and no significant differences were observed between the two mesh types (Table 2). In addition, the fibrosis scores were similar. Furthermore, the difference between the two meshes in giant cell appearance did not reach significance, although the lightweight mesh was associated with a 2-fold higher rate of nerve damage.

DISCUSSION

When a patient reports chronic pain after inguinal herniorrhaphy, neurectomy is the eventual solution if previous steps in the algorithm have failed. Selected

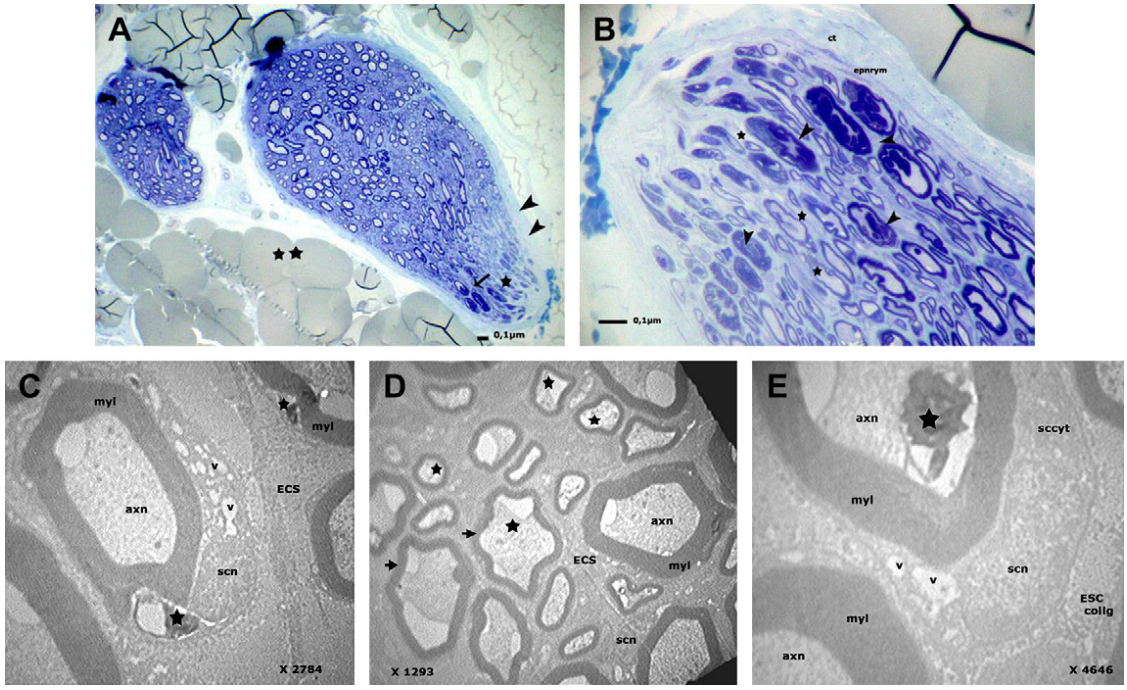

FIG. 2. Light microscopy (A), (B) and electron (C), (D), (E) micrographs of an intact nerve after heavyweight polyproylene mesh application; (A) *arrow heads*: mildly thickend perineureum, *star*: focal degeneration areas; *arrow*: axonal degeneration and myelin sheath degeneration; *two stars*: lipid cells; (B) *ct*: connective tissue; *star*: thinn myelin sheath; *arrowheads*: thickened and irreguler myelin sheat and axonal degeneration (toluidine blue; bar: 0,1 µm). Electron micrographs: *axn*: axoplasma; *myl*: myelin sheath; *unmyl*: cluster of unmyelinated fibers; *ECS*: extracelluler space; *scn*: schwann cell nucleus; (C) *star*: lamellar debris within axoplasma; *v*: vacuolization of schwan cell cytoplasm (×2784); (D) *stars*: contracted and degenerated axoplasm; *arrows*: thin and irregüler myelin sheaths and dilated axoplasma (×1293); (E) *star*: lamellar debris within axoplasma, *v*: vacuoles in schwan cell cytoplasm, *ECS collg*: collagen fibers in extracelluler space (×4646). (Color version of figure is available online.)

patients have been reported to benefit from inguinal neurectomy [16–22]. However, it is surely better to take protective measures against postoperative pain during the primary herniorrhaphy itself. Using the correct mesh with proper placement is a key technique; however, objective histopathologic changes can still be observed, even after a successful repair.

Demirer *et al.* found that mechanical compression from a polypropylene mesh could cause microscopic and ultrastructural changes in the ilioinguinal nerve [15]. Ozkan *et al.* reported that polypropylene and polytetrafluoroethylene meshes induced latency in the electromyography when they were wrapped around the nerve [23]. However, the findings of Karakayali *et al.* were rather contradictory; their prospective, randomized clinical study of motor conduction (using electromyelography) in the ilioinguinal nerve found no differences in Lichtenstein *versus* Shouldice repair groups after 1 year [24].

Our study of the effect of mesh on ilioinguinal nerve is consistent with Demirer's findings that mesh causes perineural thickening, axonal dilatation and damage to myelinated fibers [15]. However, the earlier study did not specify the properties of the material used in the mesh. In fact, the question of which mesh to use is still debated. We have examined this issue and reached results that conflict somewhat with findings from previous papers. It is generally accepted that lightweight meshes result in better healing and less foreign-body reaction [25–28]. However, it is worth remembering that the term "lightweight" may be used inconsistently among studies. Materials may also vary; some light meshes are pure polypropylene (as in this study), and some are pure polyester, whereas composite meshes combining polypropylene with absorbable materials are also available on the market.

When meshes are categorized by density, a mesh with density > 100 g/m^2 is accepted as heavy, whereas a 35–50 g/m^2 density is classified as lightweight [29]. Several recent controlled clinical studies have suggested that lightweight meshes may improve patient comfort [25–27]. Objective findings in favor of lightweight meshes have also been obtained from laboratory experiments [29]. In contrast, Weyhe and colleagues claimed that a lower weight mesh does not correlate with a decreased biological response [30]. This was also the case in our study. The lightweight mesh caused a somewhat modest fibrosis but did not generally provoke a milder response. This finding is difficult to explain based on our current knowledge.

As a mesh property, it is possible that the importance of weight is overemphasized. As a matter of fact, only

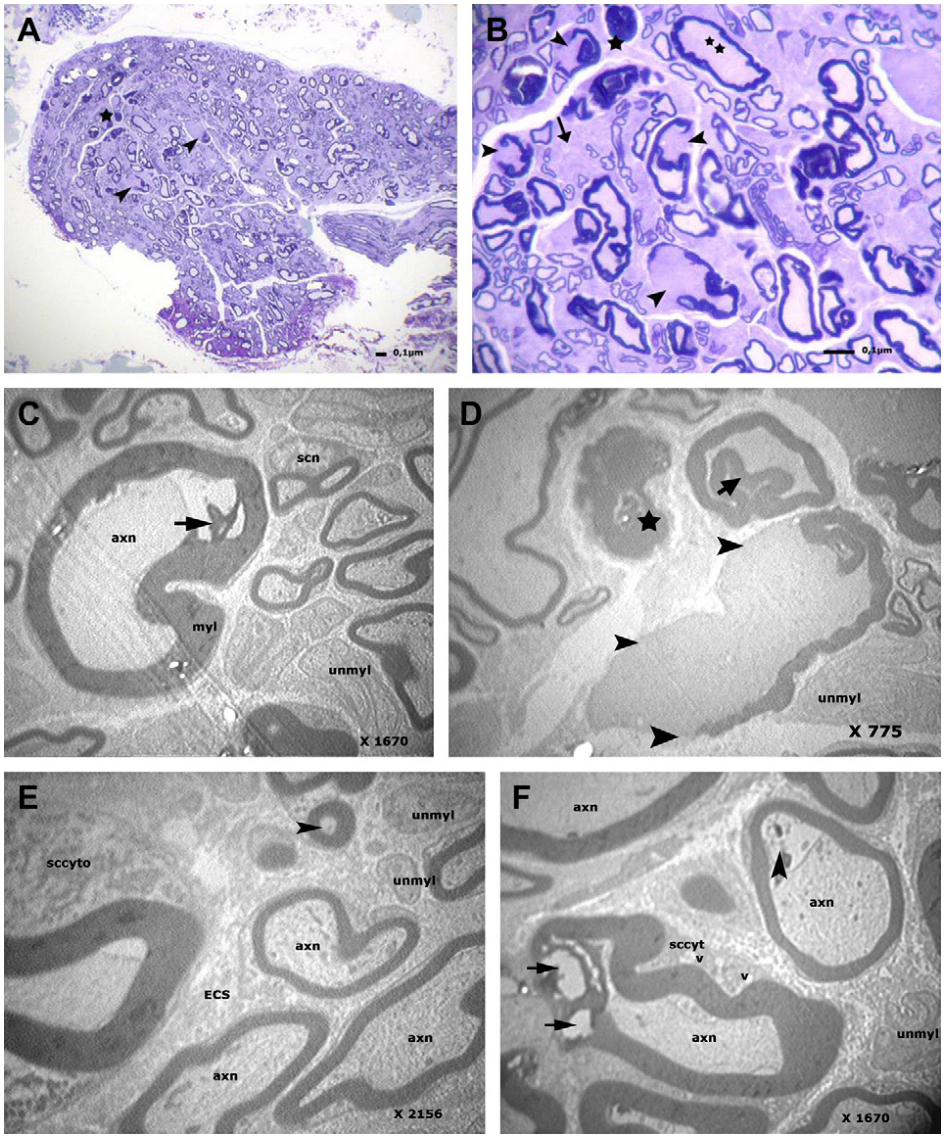

FIG. 3. Light microscopy (A), (B) and electron (C), (D), (E), (F) micrographs of an intact nerve after lightweight mesh application; (A) and (B) *arrowheads*: breakdown of myelin sheath; *star*: axonal degeneration; *two stars*: axonal swelling and dilatation (toluidine blue; bar: 0,1 μm); (C), (D), and (E) *axn*: axoplasma; myl: myelin sheath; *unmyl*: cluster of unmyelinated fibers; *ECS*: extracelluler space;; sccyt: schwan cell cytoplasm; (C) *arrow*: lameller debris within axoplasma (×1670); (D) *star*: degenerated axon and myelin; *arrowheads*: breakdown of myelin; *arrow*: redundant myelin loops (×775); (E) *arrowhead*: degenerated axon (×2156); (F) *arrows*: vacuolisation and consequent delamination of myelin lamellae; *arrowhead*: focal degeneration of the axoplasmic cytoskeleton (×1670). (Color version of figure is available online.)

small differences exist between heavy and light meshes in practice [31]. A 7.5 × 15 cm standard polypropylene mesh for an inguinal hernia repair weighs only 1.125 g, whereas the weight of a light polypropylene mesh of the same size is 0.375 g. The difference is 0.75 g. This weight difference is a negligible burden for a 70 kg person, for whom one leg alone weighs 12,600 g. The weight difference in this study, which used 2 × 1 cm meshes, was 0.0014 g. However, we should remember that any small differences between the two mesh types still reflect a 3-fold increased load on an index area or nerve segment.

Pore size may be more important than the weight, and meshes with large pores have been shown to provide better results in clinical and experimental settings [25–28]. In this context, the main criticism of Weyhe's study was that the mesh they used was lightweight but microporous [32, 33]. It has been suggested that large pores induce better healing. The lightweight mesh in our study was also a large-pore material. The only argument for a poorer result after placement of a large-pore mesh use was put forth in a very recent review: a lightweight macroporous mesh may result in better tissue in-growth but may have a higher risk of adhesions, whereas a heavyweight mesh may have a lower risk of adhesions but decreased tissue integration [34]. Although this proposal was put forth with regards to peritoneal adhesions, an analogous situation

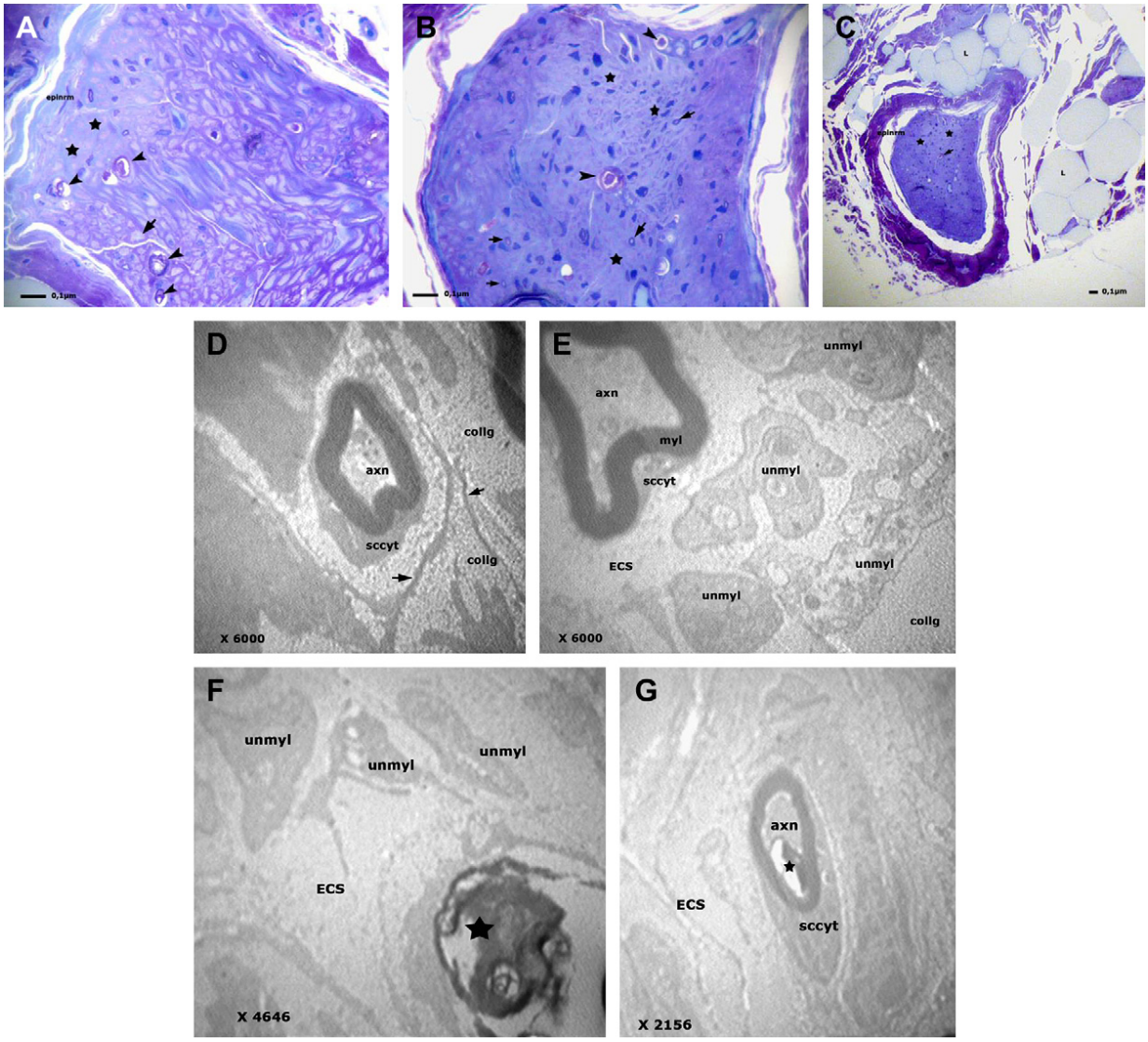

FIG. 4. Light microscopy (A), (B) and electron (C), (D), (E), (F), (G) micrographs of a cut and ligated nerve after lightweight mesh application; (A), (B), and (C) *epinrm*: epineurium; *arrowheads*: degenerated axon and myelin sheath; *arrows*: schwann cell nucleus; *star*: degeneration regions (toluidine blue; bar: 0,1 μm); (D) *axn*: axon; *sccyt*: schwann cell cyoplasm; *collg*: collagen fiber depositions; arrows: fibroblast extensions (×6000); (E) *axn*: axon; myl: myelin; *unmyl*: degenerated clusters of unmyelinated axons; *sccyt*: schwann cell ctyoplasm; *collg*: collagen deposits (×6000); (F) *star*: degenerated axon and myelin debris; *unmyl*: degenerated clusters of unmyelinated axons; *ECS*: widening of extracellular space between the axons (×4646); (G) *axn*: axoplasma; *star*: myelin debris within axoplasma (×2156). (Color version of figure is available online.)

may be valid for mesh–nerve interactions. The more frequent histopathological changes seen after placement of lightweight mesh could be a consequence of the same mechanism.

Although some of the microscopic changes mentioned above developed after mesh placement, we cannot say that the primary cause of pain after inguinal hernia repair was due to these findings. However, one condition has been shown to be directly related to postoperative pain: neuroma formation. A painful neuroma is an often debilitating sequela of peripheral nerve injury. The exact pathophysiology of this condition is poorly understood, but it is speculated that nerve endings try to find and reconnect with their distal stumps after trauma [35]. Cutting the nerve can result in a neuroma if no protective measures are taken in advance. It was observed in the present study that mere ligation of

TABLE 1

Incidence of Neuroma Formation in Three Groups

Neuroma formation	G1	G2	G3	Overall
Standard mesh	-	62.5%[a]	55.6%[b]	40.0%
Light mesh	-	25.0%	88.9%[c,d]	40.0%

[a]G2 *versus* G1; $P < 0.05$.
[b]G3 *versus* G1; $P < 0.05$.
[c]G3 *versus* G1; $P < 0.001$.
[d]G3 *versus* G2; $P < 0.05$.

TABLE 2

Fibrosis Score and the Presence of Giant Cell

Variable	Standard mesh	Lightweight mesh	*P*
Fibrosis score	1.4 ± 0.8 [0–3]	1.2 ± 0.7 [0–2]	0.225
Presence of giant cells	19.0%	38.1%	0.344

the proximal end of the nerve after division could not prevent tissue damage. This finding underlines again the importance of implanting the nerve end into the muscle after dividing it, as was previously suggested by herniologists and neurosurgeons [5, 36].

Amid suggested that, when necessary, regional nerve division should be performed at a point lateral to the deep inguinal ring [5]. Several investigations into the protective effect of cutting the nerve have described nerve division lateral to the ring such that any contact with the mesh is avoided [37]. However, an overview of the causes of hernia recurrence after Lichtenstein repair performed by the Lichtenstein Hernia Institute recommended that the mesh size be enlarged to 7.5 × 15 cm, thus extending across the lateral aspect of the deep ring toward the iliac crest [38]. This area is beyond the surgeon's direct vision in most cases, and it would not always be possible to keep the proximal cut end of the nerve from contact with the mesh. Consequently, implantation of the nerve ending into the internal oblique muscle should be considered whenever the nerve is transected in an area that will be covered by the mesh afterwards.

Although the results of this experimental study cannot extrapolate the reason of postherniorrhaphy chronic pain completely, it seems that light meshes could not provide a protection for subjects whose nerves were injured during surgery. Ligation of the cut end of the nerve was also not helpful. In conclusion, nerve protection may still be the best method for achieving a postoperative period that is free of neurologic complaints. The merits of implantation of the nerve ending into the muscle should also be reconsidered.

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