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Descriptive Histology of Different Types of Nerve Grafts in Rabbits

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ABSTRACT

This work was designed to evaluate and compare histologically between various types of nerve grafts; autograft, collagen tubes (natural polymers), and a cellular epineurial nerves sheaths (biological canduits), included a cellular rabbits nerve graft and a cellular rat nerve graft.

Forty local breaded rabbits were used and divided into four groups, and then subdivided into two subgroups (a total of 8 subgroups). Each subgroup contained five rabbits. Two times were used for animal scarifying and that were 45 days and 3 months post operation. All animals were undergone surgical operation on the sight thigh and sciatic nerve was sectioned at the mid thigh region. Segments of 8-10mm were sectioned and the gap was replaced with previous graft types. All rabbits were kept survive to the end of experiments. Comparison was made between the right (experimental) and left (control) sides. Samples were taken from proximal, middle, and distal stumps to evaluate the regeneration process.

All tissue samples were examined under light microscope. The histological results clarify an increase in amounts of fibrous tissues whether intrafascicular or extrafascicular. Also, there was an increase in epineurium thickness in the distal segments for both periods. A significant diameters decrease of nerve fibers of all experimental groups of both periods was observed compared to the control. A significant reduction in myelin thickness in the proximal and distal stamps was also obvious.

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Introduction:

The basic subunit of any peripheral nerve is the axon; the axon is an extension of the nerve cell body. Histologically, the axon may be seen as several distinct components, the center of each axon is composed of axoplasm, which is the cytoplasmic extension of the nerve cell body [(1)]. Axoplasm comprises several physiologically distinct zones that aid in transport of nutrients and essential biochemical components from the nerve cell to the terminal axon and neuromuscular terminals. The cell membrane surrounds the axoplasm and is referred to as the axolemma. Surrounding this axoplasmic unit is the Schwann cell, the Schwann cell may invers one or more axoplasmic subunits [(2)].

The peripheral nerve is represented by nerve fibers made by axons and the surrounding Schwann cells, the noble components of the nerve, while the stroma is composed by several connective tissue elements (perineurial cells) are peculiar of the nerve[(3)].

Treatment of peripheral nerve injuries is considered as challenge procedure. In the past there was no definite time of treatment. A lot of cases with peripheral nerve injuries either missed the diagnosis or found no treatment, with advent of microscope and development of microsurgical instruments, the era of microsurgical nerve reconstruction has been developed[(4)]. Surgical causes of peripheral nerve injuries include acute and chronic causes [(4)].

The aim of the present work was to compare on histological basis between different types of nerve grafts; autograft, collagen tubes (natural polymers), and a cellular epineurial nerves sheaths (biological conduits) experimentally in two periods of time, 45 days and three months post operation in rabbits.

Materials and Methods

Rabbits of local breaded type weighing (1-1.5) kg were selected and divided into four groups (A, B, C and D) and treated as follows:-

Group (A) was for autograft transplant

and subdivided into A_1 and A_2.

Group (B) selected for collagen conduit graft and subdivided into B_1& B_2.

Group (C) was for a cellular rabbit nerve graft and subdivided into C_1 &C_2.

Group (D) was for a cellular rat nerve graft, and subdivided into D_1 & D_2.

Each subgroup contained five rabbits. Subgroups (1) from each group were scarified 45 days post operation, and subgroups (2) were sacrified three months post operation. Animals were anesthetized by I.M. injection of katamine and xylazine in a ratio of 3:1.

Sciatic nerve of the right thigh was exposed in each animal by dissection of the posterior aspect of the thigh from the sciatic notch to the popliteal region, and after careful separation of the skin and underlying fascia using surgical instrument, 8-10 mm of sciatic nerve was sectioned at the middle third of thigh. The sectioned nerve was either restored as an autograft in group (A), or was replaced by collagen conduits in group (B) or grafted by a cellular rabbit sciatic in (C) or by a cellular rat nerve in (D).

All surgical procedures were performed under sterilized conditions using eye loop of (4x) magnification. Nerve suturing was made using 8/0 vicryl absorbable sutures. Fascial suturing was made with 3/0 catgut, while skin suturing was made using 3/0 black silk sutures. Animals were housed post operation in cages with normal exposure to day light/night and good feeding and drinking.

1- Autograft surgical procedure in groups (A_1 and A_2).

8-10 mm section of sciatic nerve was resutured using 8/0 vecryl absorbable suture.

2- Collagen conduits procedure in groups (B1 and B_2).

A bioresorbable collagen membrane for guided tissue regeneration type (B and B italy) was manually rolled to form a tube with a length of 1.5 cm to bridge the gap between the stamps.

3- A cellular rabbit procedure in groups (C_1 and C_2)

A total of 10 sciatic nerves were removed from white new Zealand rabbits placed in petridish, made a cellular following Marian Sondell

protocol [5]. 1-1.5 cm was taken to bridge the sciatic stumps in group C_1 and C_2.

4- A cellular rat procedure in groups (D_1 and D_2)

The procedure in (3) was followed with the exception of using a piece of sciatic nerve from white albino rats.

Animals dissection:

All animals were survived to the end of experimental periods, and then sacrified using intramuscular injection of katamine, then slaughtered to take the samples from. Three specimens were taken; one from three millimeters above the proximal stump, the second from the middle of graft, and the third was taken three millimeters below the distal stump.

Tissue preparation for light microscopy

Samples removed from the animals were immersed in 10% formalin for 24 hrs, dehydrated in graded alcohols, cleared in xylene and then blocked in paraffin wax. Sections were made using rotary microtome, dewaxed in xylene. Sections were rehydrated through graded alcohols, stained with H & E as a routine stain, Van Geissen stain for connective tissue, and periodic acid Schiff (PAS) for endoneurial tubes. Sections were then dehydrated, cleared in xylene and mounted with Canada balsam.

Statistical analyses"

Unpaired T. test was used to compare the mean and standard deviations of the data, Spss version 17 was used (6) to determine the significance differences between variables.

Results and Discussion

Descriptive histology:

A- **Control group**: the sciatic nerve trunk contains three main fascicles which were, the large one was the tibial nerve, the medium was common peroneal nerve, and the small, was sural nerve. Sometimes it has four fascicles. These fascicles were surrounded with an epineurium ranged (112-126) um thickness with little amount of adipose tissue, small sized blood vessels and dense strips of connective tissue with traces of collagen fibers. Each fascicles has

homogenous distribution of nerve fibers.

B- **Experimental groups (45 days and three months groups):**

1- Autograft group (A_1 and A_2)

Proximal part: in A_1 there was a homogenous distribution of myelinated fibers with a uniform epineurium thickness (180-210) um. while in A_2 group, there was 2-4 trunks vesicles, a well organized distributed nerve fibers, with some fibrosis, and an epineurial thickness ranged (140-180) um (figure -1).

Middle part: A_1 group shows either no definite separation of the trunk from the surrounding epineurium or with thick epineurium, there was incorporation of fibrous tissue and lymphocytes infiltration. In one case there was muscle tissues interapted with the nerve trunk.

In A_2 group, there was no definite separation between epineurium and nerve trunk.

Distal part: A_1 group shows that myelinated fibers reached to the distal stump, a well-organized and distributed myelinated fibers with the presence of fibrous tissues. Also, the presence of extramini nerve fibers. Epineurium thickness was (370-410) um.

A_2 group shows irregular distribution of myelinated axons, with the presence of extramini irregular fascicles. Epineurium was moderate to well organized with a thickness of (120-280) um. fibrosis appeared in one case, and the presence of empty cavities was clear in others, which can be due to degenerated nerve fibers (figure -2).

2- Collagen groups (B_1 & B_2):

Proximal part: in (B_1) group, there was little adipose tissue deposition in the epineurium which has a thickness of (132-160) um and area of fibrosis among the nerve bundles. In some area group of axons with some blood vessels were found in the epieurium.

Group (B_2) shows a homogenous distribution of myelinated nerve fibers with few fibrosis area, and increase in epineurium thickness ranged (190-214)

um, (figure -3).

Middle part: in (B_1), a single trunk fascicle was appeared in all cases. Thick epineurium and fibrosis area especially collagen fibers were found. Degenerated nerve bundles were found in the epineurium with the presence of myelinated axons in some parts.

A single irregular nerve trunk, was found in (B_2) group. In other cases nerve bundles were found surrounded by adipose tissue. Most sections demonstrated small diameter myelinated axons.

Distal part: in group (B_1) myelinated axons were seen with thick epineurium and area of fibrosis. A well marked bundles of fibrous tissue located within the nerve trunk, which caused misdiagnosis as a nerve bundle were clearly seen. Epineurium thickness was (210-250) um.

In B_2 group, separated fascicles with connectives tissue were seen, some of which with fibrous tissue under higher magnification. Myelinated axons reached to this part, some of which were mature axons. Areas of fibrosis were scattered between the nerve fibers, epineurium thickness range (250-290) um. (figure -4)

3- A cellular graft rabbit groups (C_1 & C_2):

Proximal part: sections of C_1 group demonstrated a uniform epineurium thickness (100-154) um. A well-organized distribution of myelinated axons (figure -5). While sections from (C_2) group revealed of nerve trunk which was composed of 2-4 fascicles, a homogenous distribution of small and large diameters of nerve fibers, with almost uniform epineurium thickness of (110-158) um.

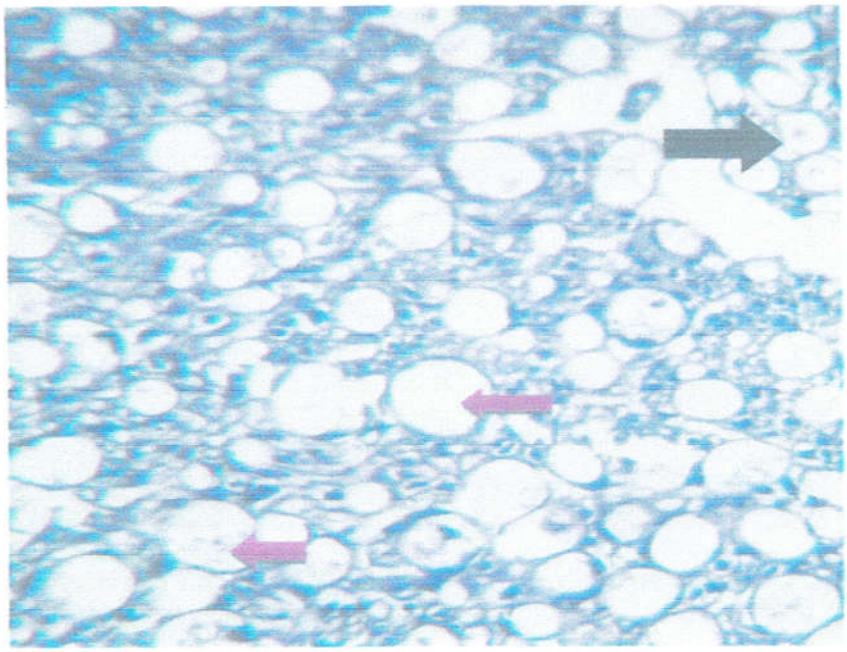

Figure -1-: Autograft group (A_1), traces of myelin (red arrow), and myelinated fibers (black arrow) after 45 days (H & E, 40x)

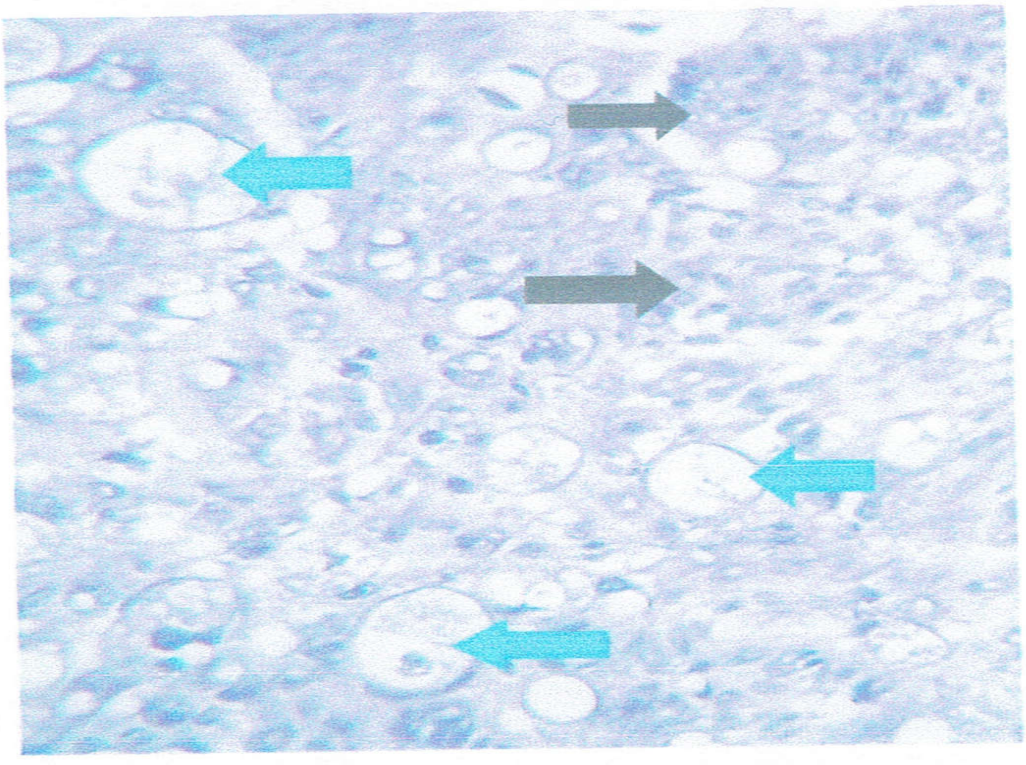

Figure -2-: Autograft group (A_2) after 3 months shows large endoneurial tubes merging of myelinated axons (blue arrows), and extramini and deformed nerve fibers (black arrow) (PAS stain, 40x)

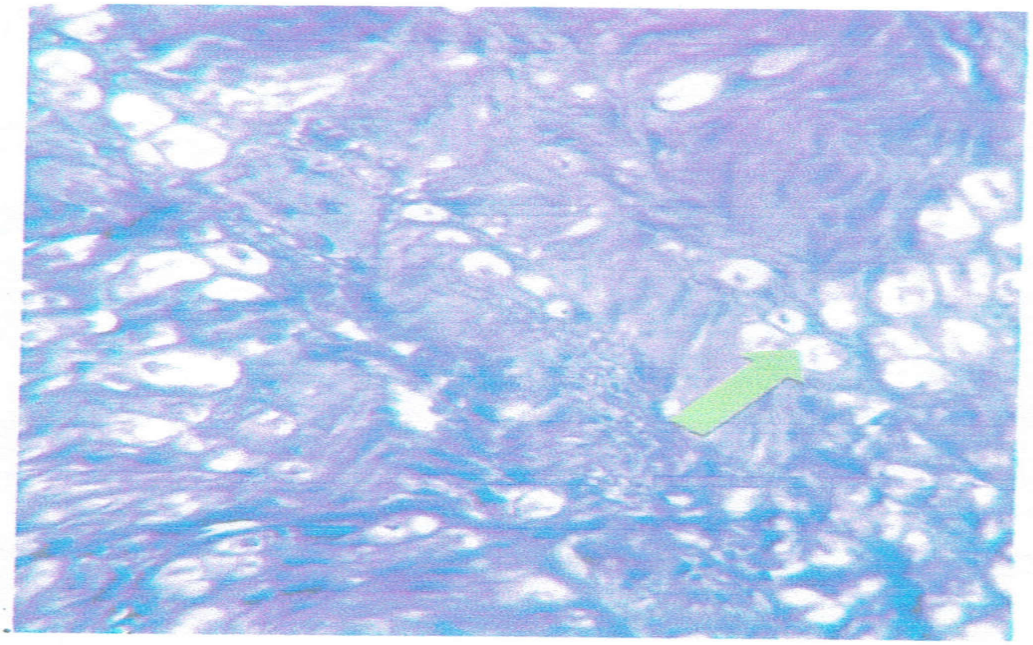

Figure -3-: Collagen group (B_1) after 45 days shows myelinated axons (green arrow), (H & E, 40x)

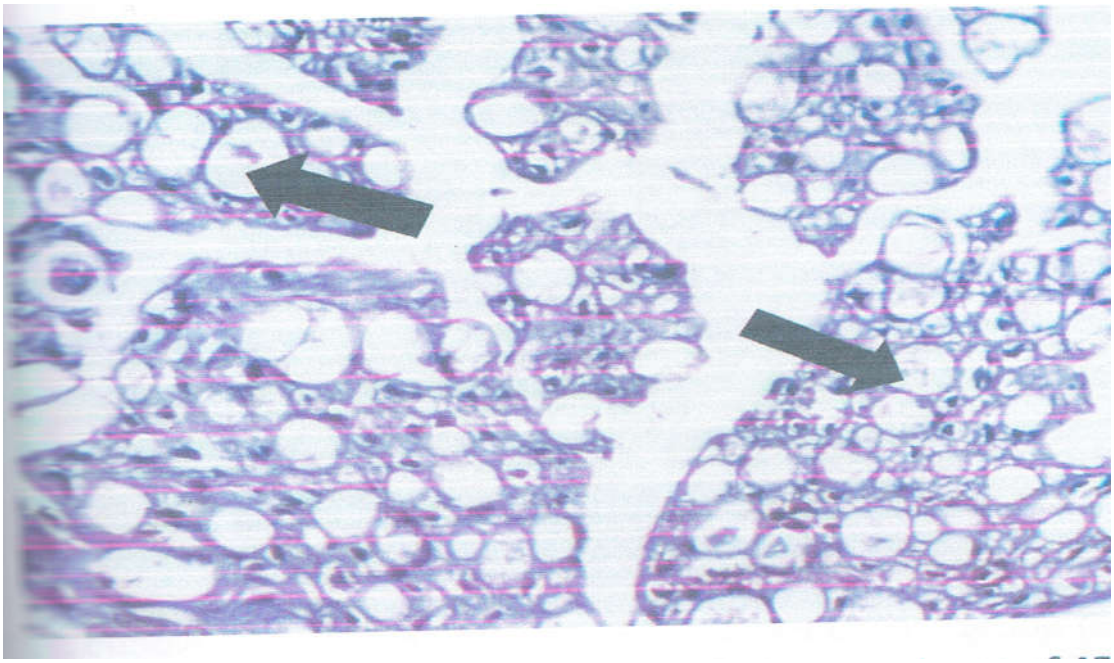

Figure -4-: Collagen group (B_2), after 3 months, myelinated axons (black arrow), (H & E, 40x)

Middle part: C_1 group graft appeared as a single fascicle trunk with thick epineurium and few scattered blood vessels, small nerve fascicle within area of fibrosis was seen in one case.

In C_2 group, all cases were with single trunk fascicle with no definite obvious separation of the nerve trunk from the epineurium, except in two cases (40%), there was a poor moderate developed nerve fascicle within the trunk, and a well-recognized epineurium around the trunk. In general, area of fibrosis were seen in between the nerve fascicles. Also section of empty endoneurial tubes invaded with fibrous tissues and lymphocytes infiltrations were seen.

Distal part: the axons in group (C_1) have reached to the distal stump, in addition to degenerated large vacuoles, fibrosin, were obviously seen. Epineurium thickness ranged (300-324) um lymphocytes infiltration was also clear.

In (C_2) group, the epineurium thickness was (200-280) um myelinated axons have reached to this part, but the axons were small, with thickening of connective tissues in some cases. Large distribution of fibrous tissue, even there was complete fascicles of fibrous tissue in the trunk (figure -6).

4- A cellular graft rat group (D_1 & D_2):

Proximal part: in (D_1) group, epineurium thickness ranged (300-320) um. sections of this group appeared normal with traces of fibrosis in some cases (figure -7). In-group D_2, the sections were with a homogenous population of myelinated and some unmyelinated axons. The trunk was surrounded with a thick epineurium (160-190) um. few areas of fibrosis were seen.

Middle part: in (D_1) group, invasion of fibrosis was seen either in the trunk or as a thick epineurium. Blood vessels were seen in the epineurium as well as myelinated axons.

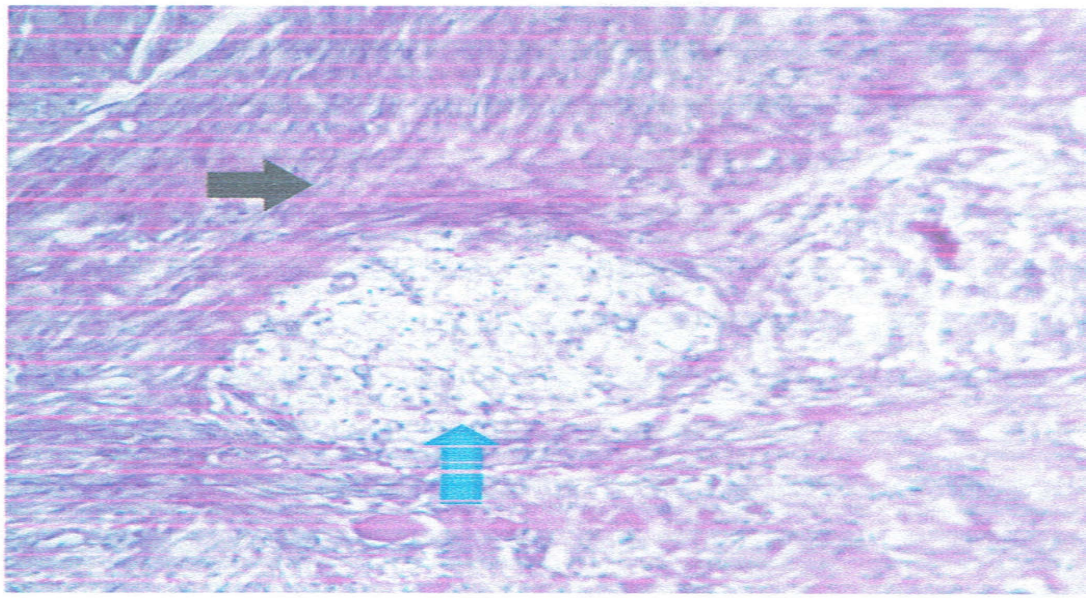

Figure -5-: A cellular graft rabbit group after 45 days, shows a single nerve fascicle (blue arrow) within fibrosis (black arrow). (H & E, 10x)

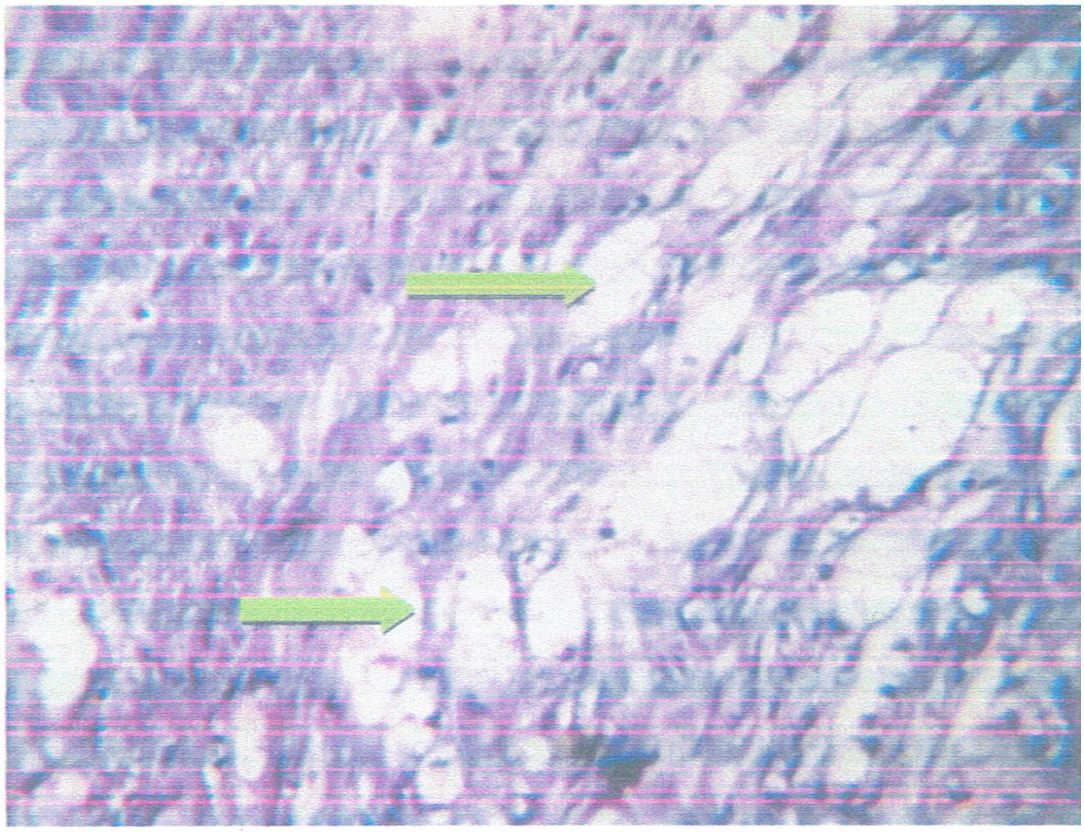

Figure -6-: A cellular graft rabbit group after three months shows myelinated axons within epineurium. (H & E, 40x)

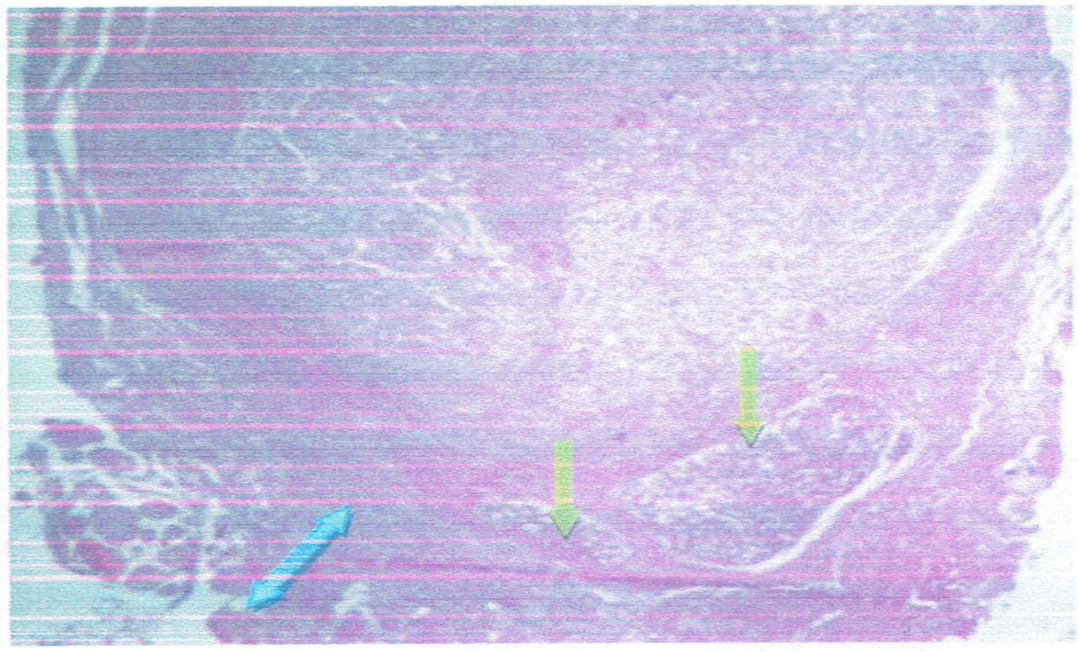

Figure -7-: A cellular graft rat group after 45 days shows, nerve bundles (green arrow) within thick epineurium (blue arrow). (VG, 10x)

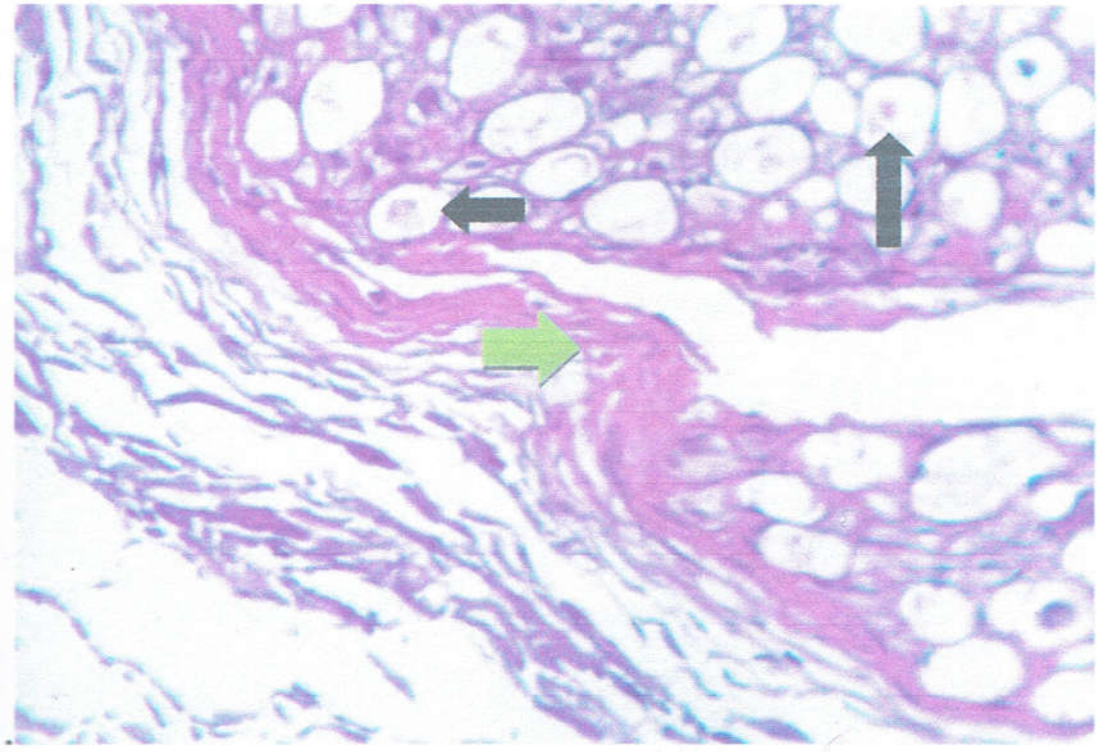

Figure -8-: A cellular graft rat group after 3 months shows, myelinated axons near epineurium (black arrows), Collagen fibers deposition in thick epineurium (green arrow). (VG, 400x)

In-group (D_2), the trunk was seen with a single fascicle, except in one case was with two fascicles. Epineurium was thick, but in some cases, there was no definite separation of the epineurium from the trunk.

Distal part: axons in (D_1) group have reached to the distal stump, however, there were degenerated large vacuoles, fibrosis, and thick epineurium (300-324) um. lymphocyte filtration was also seen.

Sections of D_2 group demonstrated thick epineurium with massive penetration of adipose tissue. Epineurium thickness ranged (240-320) um. In all cases, there was no definite difference between the epineurium and nerve trunk except in one case only. Few myelinated axons were defected and small size axons in all sections were present. Intrafascicular fibrosis around the endoneurium was clearly obvious (figure -8)

Discussion

The effect of nerve injury repair and their subsequent effect on neuron regeneration are very difficult, specially when it is concerned with human situation, because of the difficulty of obtaining tissue samples, and to elucidate the related inflammation, and then the repair stages without causing great damage to the surrounding tissues and associated nerves. Therefore, most nerve injury and regeneration processes have been conducted on lab animals, mostly in rats [(7)].

When axons are detached from their cell bodies in the distal part there will be a fragmentation stage through a self-destruction (i.e, Wallerian degeneration), because of the disconnection of axons, myelinating Schwann cells turn into immature phenotype, and proliferation start in order to help myelin degeneration [(8)].

Wallerian degeneration cause a strong neuroinflammatory response in which Schwann cells are believed to may important role in the production of inflammatory cells such as macrophages lymphocytes, neutrophils, promote the direction of macrophages toward the injured nerves, in addition to the accumulation of mast cells in injured nerve endoneurium [(9)]. The

injury itself leads to a breakdown of the blood-nerve barrier, and then fibrinogen leak to the place which is converted into fibrin [10]. With all these events, two to three weeks after injury the inflammatory processes are turned off and macrophages and other inflammatory cells are eliminated [9].

Histologically, an increase amount of fibrous tissue (i.e, intrafscicular or outer fascicular in the epineurium and mainly in the distal segment for the 45 days group and specially the three months groups, particularly in C_2 and D_2 groups starts. This is related to the previous detailed steps of Wallerian degeneration. Therefore, the endoneurial tubes remain denervated for prolonged periods. After 3-4 months the endoneurial tubes remain denervated for long periods and then start to shrink, the endoneurial sheath thickens secondary towards collagen deposition along the basement membrane of Schwann cells and the outer surface. If the endoneurial tubes does not receive a regenerating axons, then ultimately fibrosis start to close it [11].

Epineurial sutures do not prevent nerve tissues outgrowth or ingrowth of scar tissue from the repair sites, and many of these repairs are unsatisfactory except in children [12]. More over the undesirable cell reactions in the early regeneration stages of heterogenous and homogenous grafts may causes small to large amount of fibers to enter the grafts [13].

These results were in agreement with Guo et.al [14], and with a study of Colin and Donoff [15] and with the Holland, et.al, where they stated the outgrowth of many connective tissue in the distal stump [16]. Fibrous tissue was found in different amounts in the proximal part in various degrees and in all study groups. Retrograde axonal degeneration in the proximal nerve stump may extend for several millimeters or centimeters following severe nerve injuries. The remaining axons in the proximal stump may also show a diameter reduction. These findings are in agreement with Ronald et.al, study [17].

In conclusion, histologically, all experimental groups of 45 days and

three months exhibited variable degrees of nerve regeneration. Bridging the gap of transected nerve was facilitated by conduits and collagen regeneration of nerve trunk and characterized by various degrees of fibrous tissues. Collagen conduits showed good traversing of new axons in relation between proximal and distal stumps compared to autograft. And finally, the regeneration process was related to the following points; steps of Wallerian degeneration, sprouting, guidance for regeneration, Schwann-lymphocyte-macrophage interactions, neuron death, extracellular matrix and inflammation.

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