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EVALUATION OF BIODEGRADABLE POLYGLYCOLIC ACID MESH SCAFFOLD AS A NERVE CONDUIT FOR FACIAL NERVE DEFECT IN RABBITS

By

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ABSTRACT

The aim of this study was to evaluate efficiency of a biodegradable polyglycolic acid mesh (PGA) as a nerve conduit in facial nerve defect in a rabbit model. Sixteen healthy adult rabbits were randomly divided into two equal groups (n=8). In group A, a 0.5 cm of left dorsal buccal branch of facial nerve was transected and coaptated using PGA mesh conduit while in group B, (Control), which had a 0.5 cm defect without anastomosis. Clinical and histopathological evaluations were done. In group A, clinical evaluation showed normal symmetry of whisker movement at 16 weeks postoperatively while in group B, there was absence of whisker during the study period. Gross pathological examinations showed complete absorption of the mesh and reconnection of the nerve stumps while group B, showed no reconnection with neuroma formation at the proximal stump. Histopathological finding in group A, showed good myelination of regenerated nerve fibers and deposition of collagen fibers while in group B, showed degenerative nerve fibers and scar tissue formation. Ultrastructural of group A, showed good myelination, presence of active Schwann cells and deposition of collagen fibers while in group B degenerated axon, degenerative Schwann cells and heavy deposition of collagen fibers were noticed. In conclusion. The PGA mesh conduit is acceptable for facial nerve regeneration in rabbits.

Keywords:

Polyglycolic acid (PGA) meshes, histopathology, electron microscopy, facial nerve, rabbits, nerve regeneration.

INTRODUCTION

Facial nerve is one of peripheral nervous system that commonly exposed to injury in animals, the most commonly causes of facial nerve injuries are idiopathic facial nerve paralysis, buccal branch injury from prolong lateral recumbency, rough handling, automobile accidents or surgery such as bulla osteotomy and total ear ablation which lead to facial paralysis **(Schubert, 2013)**. Facial nerve paralysis causes absent blink reflex, lip droop, ear droop, hypersalivation, nasal deviation toward the normal side, absent palpebral reflex, absent lip movement and corneal ulceration may be present if the parasympathetic branches of the facial nerve that innervate the lacrimal glands are also affected **(Troxel, 2016)**. Facial nerve has the ability to regenerate following injury due to the ability of the Schwann cells to promote axon regrowth **(Kamijo *et al*., 2003 and Gaudet *et al.*, 2011)**. Many strategies are used to bridge nerve ends as autologous nerve grafts that widely used for nerve gap reconstruction but it has a disadvantage of limited availability of donor nerves **(Pfister *et al*, 2011)**. Poly glycolic acid (PGA)mesh isa synthetic polymer; it has been used widely into a variety of three-dimensional scaffolds for tissue engineering purposes including nerve repair applications **(Weber *et al*., 2000 and Patel *et al*., 2011)**. It is a porous biodegradable polymer allows the infiltration of oxygen to support the regeneration process, and is absorbed in the body via hydrolysis **(Costa *et al*., 2013)**. So, the present study was done to evaluate the efficiency of PGA mesh as a conduit for facial nerve regeneration in rabbits through clinical, gross and histopathological as well as transmission electron micrography.

MATERIAL AND METHODS

Experimental animal:

This study was conducted on 16 healthy adult white New Zealand rabbits (4± 0.78) months old, weighing (3± 0.18) kg from both sexes (6 males, 12 females). These rabbits were divided into two groups (n=8): group A, a 0.5 cm of the left buccal branch of facial nerve was excised and anastomosed by PGA mesh. Group B (control group), a 0.5 cm of the left buccal branch of facial nerve was excised and was left without anastomosis.

Anesthesia:

The rabbits were fasted for two hours prior to the anesthesia. The animals were anesthetized by intramuscular injection of a mixture of xylazine (5mg/ kg) (Adwia Company, Egypt) and ketamine (35mg/kg) (Alfasan International Company, Holland) according to **(Oguntoye and Oke, 2014).**

Surgical technique:

The area of operation was aseptically prepared for surgery. In group A, the skin incision was made in the left side of face below zygomatic arch extended 1cm rostral to the ear base to the mid-region of mandible then gentle dissection was performed to isolate and identify the dorsal buccal branch of facial nerve**.** Surgical excision of 0.5cm then the nerve ends were anastomosed with PGA mesh and fixed the mesh on nerve stumps by PGA suture 5\0 **(**DemeTech Company, USA) then wrapping the mesh as a tube and fixed it by 2-3 stitches of PGA suture 3/0 Fig. (1). in group B, surgical excision of 0.5 cm of the nerve and then the nerve ends were left without anastomosis.

Postoperative care:

The experimental rabbits received penicillin-streptomycin (Norbrook Company, UK) in a dose of penicillin 10.000 I.U./Kg BW and streptomycin 10 mg/kg BW for 3 days by I.M route injection. Rabbits were kept in individual wire cages for 120 days under continuous clinical evaluation.Then, Rabbits were euthanized by exsanguination after anesthesia **(Olfert *et al.*, 1993)** and subjected to gross and histopathological examination.

Clinical examination:

The rabbits were checked out for recording any surgical complication. The assessment of whisker in the left side was done weekly and compared with the right side according to **(Yildirim *et al*., 2015).**

Gross pathological examination:

The gross pathological examinations included nerve reconnection, PGA mesh absorption, change nerve thickness and presence of neuroma **(Al-Timmemi *et al.*, 2013).**

Histopathological examination:

Biopsies were collected from the buccal branch of facial nerve of rabbits. The samples fixed in 10% in buffered formalin for 24 h and stained by hematoxylin and eosin stains for routine examination **(Bancroft and Gamble, 2002)**. Axonal alignment, neural scarring, disarrangement and presence of vacuolated degenerate nerve fibers, staining intensity of Schwann cells should be evaluated **(Jasim and Alfaris, 2016).**

Transmission electron microscopy examination:

Ultra-thin sections (50 - 80 nm) were prepared then stained with heavy metals (uranyl acetate followed by lead citrate) according to **(Williams and Carter, 2009).**

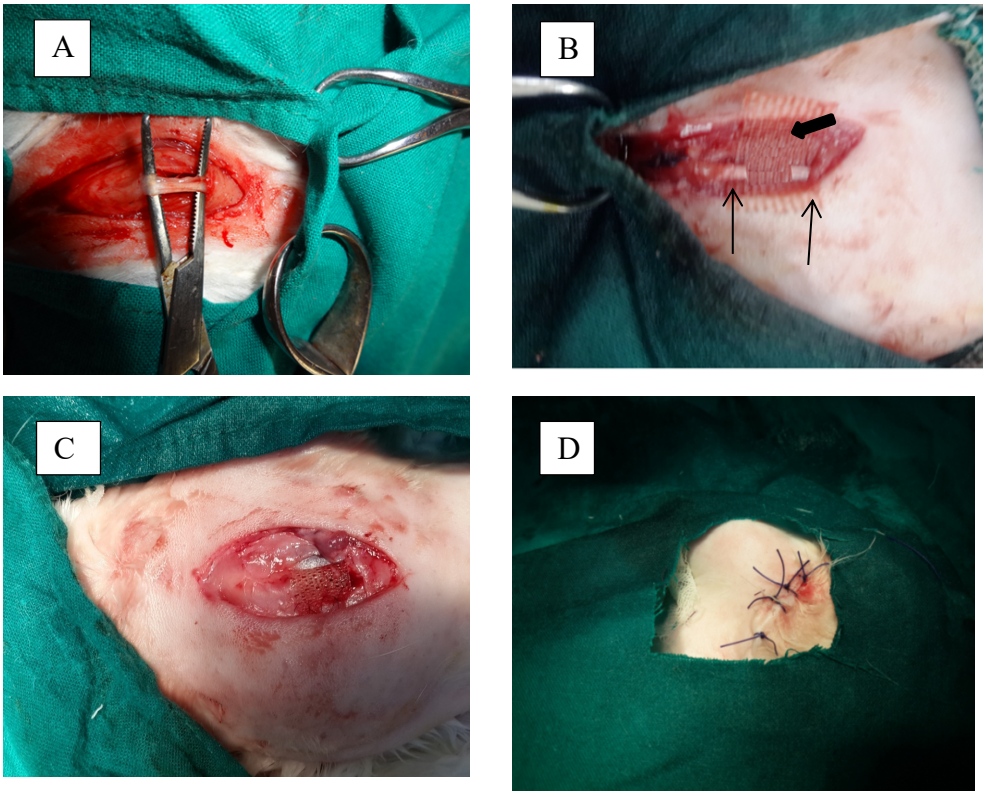

Fig. (1): Surgical operation of group A: A. Exposure of dorsal buccal branch of facial nerve. B. A 0.5 cm of nerve was removed and 1 cm PGA mesh palced under nerve (Thick arrow), a 0.25 cm under each two nerve ends (Thin arrows) and 0.5 cm under nerve gap. C. Wrapped PGA mesh as a tube-like structure and fixed it by PGA 5/0 D. Closure of skin wound after with simple interrupted suture.

RESULT

The mixture of ketamine-xylazine was safe, well tolerated and produced a satisfactory general anesthesia for performing surgery. Fifteen rabbits survived while one rabbit in group A, died at day 35 postoperative for unknown reason.

Clinical finding:

In group A, no obvious signs of whiskers movement were noticed for 5 weeks postoperatively then the sign of slight movement appeared after the 5th week and continued to the week 16. The signs of normal whiskers movement appeared after the week 16 while in the group B, complete absence of whisker movement in the left side was noticed during the full study period.

Gross pathologic finding:

The gross pathological finding in group A, after surgery showed nerve reconnection, complete absorption of the PGA mesh and fibrous tissue thickening (1± 0.2 mm) at site where the PGA mesh was implanted Fig. (2, A). In group B, the regeneration did not occur across the gap, shrinkage backward of nerve stumps, fibrous adhesion and neuroma formation at the proximal stump of nerve Fig. (2, B).

Histopathological finding:

Histopathological examination of the facial nerve stained with H&E on day 120 after facial nerve surgery under light microscopic. The samples were collected from the middle site of regenerated nerve of group A, while in group B, there was no nerve regeneration and samples collected from proximal and distal stumps. Longitudinal sections of the proximal nerve stump of the facial nerve of rabbit in group A, showed focal inflammatory cells infiltration, highly vascularized and newly formed blood capillaries, good myelination of regenerated nerve fibers and deposition of collagen fibers Fig. (3). Histopathological examination of the facial nerve of rabbit in group B, revealed degeneration of nerve fibers and few numbers of Schwann cells, focal inflammatory cells infiltration in the degenerative nerve fibers and scar tissue formation in proximal and distal stumps Fig. (4, A and B).

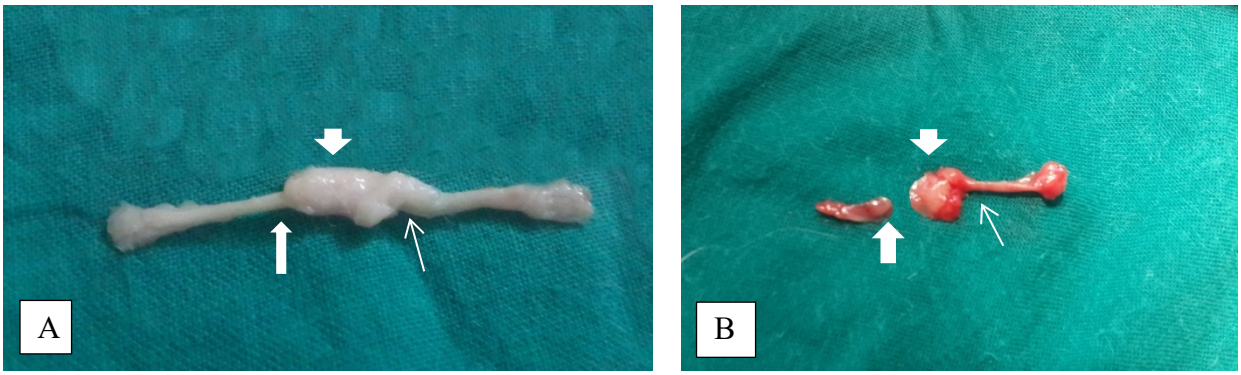

Fig. (2,A): The nerve segment in group A, showing reconnection of the proximal (Thin arrow) and distal stumps (Thick arrow) with thickened regenerated nerve middle part (Head arrow). B. The nerve segments in group B, showing loss of reconnection between proximal (Thin arrow) and distal (Thick arrow) stumps, adhesion attached to proximal stumps and neuroma formation (Head arrow).

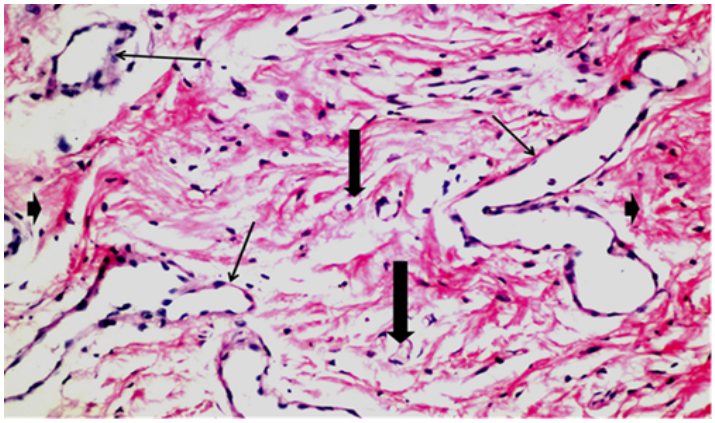

Fig. (3): Light micrographs of the facial nerve of rabbit in the group A, on day 120 postoperatively, showing highly vascularized and newly formed blood capillaries (Thin arrows), good myelination of regenerative nerve fibers (Thick arrows) and scanty of collagen fibers (Arrows head). **H&E X40.**

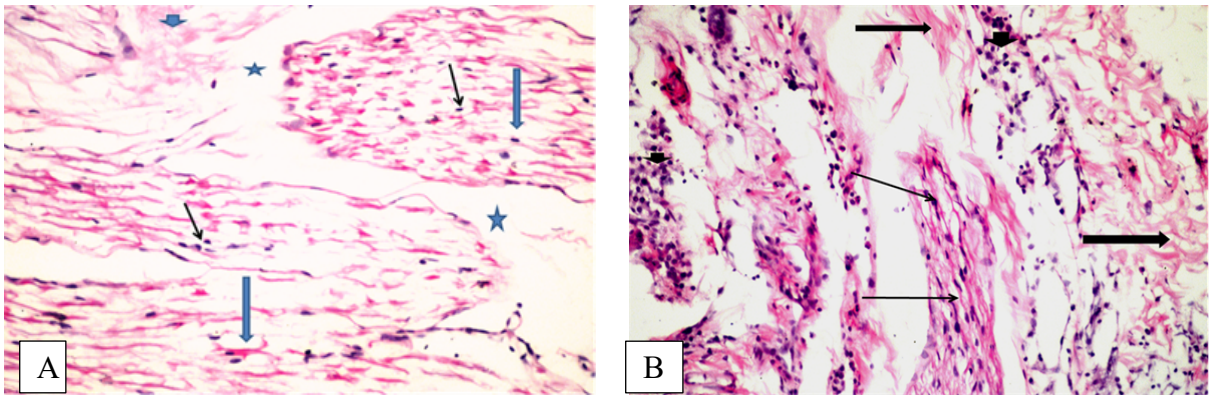

Fig. (4, A): Light micrographs of the facial nerve of rabbit in proximal segment of group B, showing degeneration of nerve fibers (Thick arrows), few numbers of Schwann cells (Thin arrows), and deposition of scar tissue within gap (Arrow head). **H&E X 40. B.** Light micrographs of the facial nerve of rabbit in group B, in distal segment showing degeneration of nerve fibers (Thin arrows), focal inflammatory cells infiltration in the degenerative nerve fibers of the bundle (arrows head) and scar tissue formation (Thick arrows). **H&E X 40.**

Electron microscopic finding:

Ultrastructural of the regenerated nerve segments in group A, showed good myelination of regenerative nerve fibers, nucleated Schwann cell and deposition of collagen fibers Fig. (5). In group B, ultrastructural of the proximal segment of facial nerve showed psychotic nucleus of Schwann cells and heavy deposition of collagen fibers Fig. (6, A) while, the distal segment showed remnant Schwann cells, and heavy deposition of collagen fibers and fibrocyte cells Fig. (6, B).

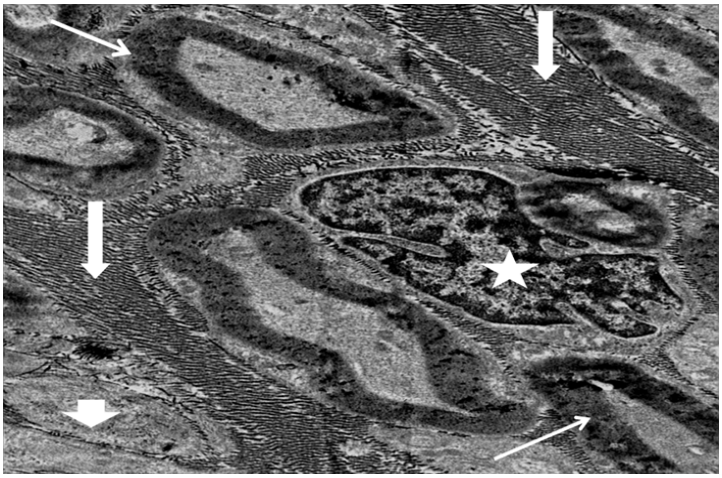

Fig. (5): Electro micrographs of the of facial nerve in group A, show good myelination of regenerated nerve fibers (Thin arrow), nucleated Schwann cell (Asterisk) and deposition of collagen fibers (Thick arrows) . **UA&LC X15000.**

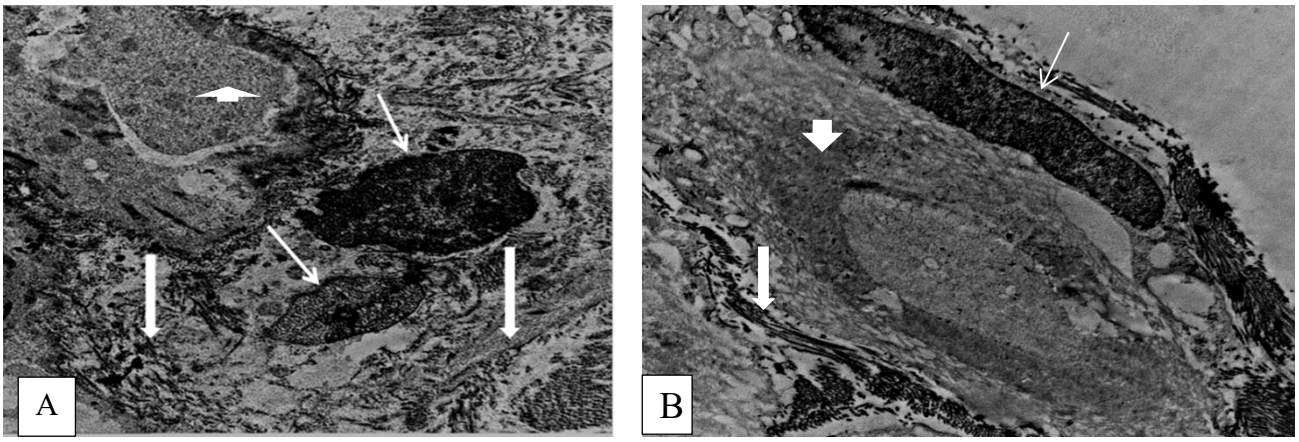

Fig. (6, A): Electro micrograph of the proximal segment of facial nerve in group B, show pyknotic nucleus Schwann cells (Thin arrows), heavy deposition of collagen fibers (Thick arrows) degenerated axon (Arrow head). UA&LC X 10000. B. Electro micrograph of distal segment of facial nerve of group B, showing remnant Schwann cells (Thin arrow), heavy deposited of collagen fibers (Thick arrow) and degenerated axon (Arrow head).**UA&LC X 10000.**

DISCUSSION

The ideal nerve conduit should be biodegradable, permeable, flexible, non-toxic, and not elicit an immunological response **(Tan *et al*., 2012 and de Ruiter *et al*., 2009)**. The present study clarified the efficiency of PGA mesh as a nerve conduit in 0.5 cm facial nerve defect in rabbits. The use of biodegradable materials for nerve reconstruction is valuable treatment for

facial nerve injury **(Navissano *et al*., 2005and Liu *et al*., 2013)**. In the present study, complete absorption of the PGA mesh at the end of experiment was seen in all rabbits of group A that result in a tensionless repair without compression on the regenerated nerve **(Dellon, 1995 and Costa *et al*., 2013)**. PGA has been exhibited biocompatibility characteristics for nerve implantation **(Weber *et al*., 2000; Wang *et al*., 2005; Hu *et al*., 2008)**. In the present study, no signs of infection were recorded which confirming the good tissue acceptance of PGA mesh. Our data are in agreement with others that used PGA alone or in conjunction with certain absorbable substance as nerve conduit for peripheral nerve regeneration **(Wang *et al*., 2005 and Yoshitani *et al*., 2007)**. Clinical evaluation of whisker is often adopted in the facial nerve models as a functional test for facial nerve healing **(Yildirim *et al*., 2015)**. A, symmetrical normal whisker was noticed in group A in contrast of group B, there was absence of symmetrical of whisker, this indicating the functional recovery of the facial nerve in group A **(Zhu *et al*., 2015)**. Gross pathological finding showed thickening of the regenerated facial nerve in group A, which could be explained as fibrous connective tissue surrounding the nerve **(Hegtvedt *et al*., 2002)**. The histopathological analysis of this thickening demonstrated inflammation, deposition of fibrous connective tissue without neuroma formation and these findings were reported in sciatic nerve defect in rats **(Costa *et al*., 2015)** who explained absence of neuroma due to formation of fibrous tissue around the nerve which preventing neuroma formation and axonal escape. Implantation of PGA mesh serve as a directional guide to facilitate functioning properly of Schwann cells migration lead to good myelination and regeneration of nerve fibers **(Hu *et al*., 2008)**. Slight inflammation in the group A, may be due to PGA mesh degradation **(Ceonzo *et al*., 2006).** Vacuolated degeneration changes in nerve fibers and scar tissue formation noticed at proximal and distal nerve stumps of the group B may be as a result of separation of proximal and distal ends. Similar changes were mentioned in rat model **(Khan *et al*., 2014; Rafee *et al*., 2017)**. These findings were explained by **(Al-Timmemi *et al*., 2013)** who reported that, the internal endoneurial fibrosis at the injured site might divert re-growing axons, scar formation, continuous degeneration and deposition of collagen induced a retardation of the recovery process. Good myelination of regenerative nerve fibers in group A, observed under a transmission electron microscope was in accordance with the observation by **(Matsumoto *et al*. 2000)**. Presence of active Schwann cells indicates regeneration of facial nerve as Schwann cells play obligatory role in axonal regrowth. Schwann cells organize themselves

longitudinally in columns called bands of Bungner providing a supportive environment and guide axons for successful axonal regeneration towards target organ **(Bunge, 1993)**. Failure of nerve recovery in group B indicating that, the failure of the neurons and Schwann cells to sustain axon regeneration **(Grinsell and Keating, 2014)**. Ultra-structural micrograph of group B revealed degeneration of axon and Schwann cells death. Death of Schwann cells lead to failure of nerve regeneration and myelination **(Winseck *et al*., 2002)**. Scarring and fibrosis at the defected site from tissue handling, the injury itself and loss of reconnection, all of these factors lead to failure of regeneration neuroma formation **(Fu and Gordon, 1995).** In conclusion that, the nerve reconstruction with polyglycolic acid mesh is economically, cheap and feasible technique providing improves the facial nerve regeneration in short nerve defect in rabbits. The main limitation of present study was absence of sequential evaluation of rabbits during study period as well as absence of electromyogram.

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