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EFFICACY OF POLYGLYCOLIC ACID CONDUIT AND ADIPOSE-DERIVED STEM CELLS ON REGENERATION OF FACIAL NERVE DEFECT IN RABBITS

By

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ABSTRACT

The aim of this study was to compare between the polyglycolic acid (PGA) conduit seeded adipose-derived stem cells (ADSCs) and empty PGA mesh on the regeneration of facial nerve defect in rabbit model. Sixteen healthy adult rabbits were randomly divided into two equal groups (n=8). In the group A, a 5 mm of left buccal branch facial nerve was transected and coaptated using PGA conduit only while in group B, the same nerve defect was established and coaptated by PGA mesh seeded ADSCs. Clinical signs and histomorphometric evaluation were done. The clinical evaluation of movement of upper lip and attached vibrissae in group B, showed significant difference ($P \leq 0.05$) compared with group A,. Histological examination in group B, the regenerated nerve fibers were well organized with good nerve architecture, abundant nerve fascicles and dense myelinated axons. Morphometrical results of the regenerated nerves fibers showed significant increase in the number of regenerated nerve fibers, myelin thickness and diameter of regenerated nerve fibers in group B than in group A, So, we can conclude that PGA mesh seeded with ADSCs provides better and significant improvement in facial nerve regeneration in rabbit's models than empty PGA mesh conduit.

Key words:

Adipose-derived stem cells, facial nerve defect, Polyglycolic acid mesh, Rabbits.

INTRODUCTION

Facial nerve is a mixed nerve provides sensory branches to the area around ear and rostral two-thirds of tongue and motor branches to the muscles of facial expression (Including muscles of eyelids, ear, nose, cheeks, and lips), the posterior portion of the digastric muscle,

lacrimal glands, mandibular and sublingual salivary glands **(Reece, 2005 and Lorenz *et al*.,2011)**. Facial nerve injuries commonly occurred in animals; in large animal, after prolong lateral recumbency or halter injuries, while in small animal occurred after surgical operations of the ear **(Schubert, 2013).** Artificial conduit is one of the most promising techniques of peripheral nervous repair as it provides good environment for nerve regeneration **(Kim *et al*, 2007 and Bakhtyari *et al*, 2009)**. Recent advances in nerve tissue engineering have greatly promoted the generation of nerve conduit implanted empty, or may be filled with growth factors, cells or fibers **(Heath and Rutkowski, 1998)**. Adipose-derived stem cells (ADSCs) are abundant, easily accessible, easily harvested, and have immunosuppressive properties and low immunogenicity **(Strem *et al*., 2005 and di Summa *et al*,2010)**. The present study investigates the efficiency of polyglycolic acid (PGA) mesh conduit and PGA mesh seeded with ADSCs on regeneration of facial nerve defect in rabbits.

MATERIAL AND METHODS

Experimental Animals:

This study was conducted on 16 healthy adult white New Zealand rabbits (4± 0.6) months old, weighing (3± 0.2) kg from both sexes (6 males, 12 females). These rabbits were divided into two groups (n=8). In the group A, a 5mm of left buccal branch facial nerve was transected and coaptated by PGA mesh conduit only. In the group B, the same surgical procedure was done but cooptation by PGA mesh seeded with ADSCs.

Isolation of Adipose-Derived Stem Cells of the Rabbits:

Isolation of adipose tissue was collected from the abdomen of rabbit after euthanasia with exsanguination after anesthesia **(Olfert *et al.*, 1993)**. ADSCs were isolated from adipose tissue and loaded in chitosan hydrogel in a dose of 5×10^6 according to **(Kingham *et al*., 2007).**

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 techniques:

After the preparation the site of surgery for aseptic operation, the incision was made in the left side of animal's face below zygomatic arch extended 1cm rostral to the ear base to the mid-region of mandible and should be large enough for good exploration buccal branch of

facial nerve, then gentle dissection was performed to isolate and identify the dorsal buccal branch of facial nerve. In group A, surgical transected of 5mm nerve segment then anastomosed of two nerve stumps 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 by 2-3 stitches of PGA suture 3/0 (DemeTech Company, USA). In group B surgical transected of 5mm nerve segment then coaptated the two nerve segments with PGA mesh and fixed the mesh on nerve stumps by PGA suture 5/0, then 1 ml of ADSCs loaded in chitosan hydrogel was put in the mesh and filled nerve gap then wrapping the mesh as a tube around gel and fixed it by 2-3 stitches of PGA suture 3/0.

Clinical Evaluation:

The clinical assessment of the left upper lip movement and vibrissae attached was done three times weekly and compared it with the right side according to **(Yian *et al*., 2001)**. A grading scale was established ranging from 0 to 3 (0, no movement; 1, slight movement; 2, moderate movement; 3, normal movement).

Histomorphometrical Analysis:

Samples were collected from the middle part of regenerated facial nerves for histological and morphological analysis. The samples were prepared and stained with toluidine blue stain according to **(Costa *et al*., 2013).** Morphometric measurements of the facial nerve were included the myelinated nerve fibers numbers, myelin sheath thickness and fiber diameter **(Watanabe *et al*., 2014).**

Statistical analysis:

The collected data were analyzed using t-test in SPSS statistical software (Version 24). Values of $P < 0.05$ were considered statistically significant.

RESULTS

Clinical evaluation:

The clinical evaluation of movement of upper lip and attached vibrissae was done weekly and recorded in (Table 1). Complete recovery was considered when the movement of upper lips and attached vibrissae was symmetrically to the contralateral side Fig. (1).

Histomorphometrical Analysis of Nerve Regenerated:

Histological examination of the group A, showed less organized regenerated myelinated axons, few nerve fascicles, less dense myelinated axons and adipose tissue infiltration between fascicles while in group B, showed well organized regenerated myelinated axons,

good nerve architecture, abundant nerve fascicles and dense myelinated axons Fig. (2). Morphometric results of the regenerated nerves fibers showed significant increase in the myelinated nerve fibers numbers in group B (3486.25± 91.23, $p<0.05$) than the group A, (2844.5 ± 85.02, $p<0.05$) Fig. (3). Myelin thickness was significantly greater in group B (2.56±0.44µm, $p<0.05$) than group A, (0.62±0.2 µm, $p<0.05$) Fig. (4). Fiber diameter was significantly higher in group B (5.89±2.1µm, $p<0.05$) than group A, (2.56±1.03 µm, $p<0.05$) Fig. (5).

Table (1): Functional evaluation of upper lips and vibrissae movement for left side of experimental animals in both groups (A and B).

	Score0	Score1	Score2	Score3
Group A	**1-5 weeks**	**5-9 weeks**	**10-16 weeks**	**Above 16 weeks**
Group B	**1-4 weeks**	**4-9 weeks**	**10-14 weeks**	**Above 14 weeks**

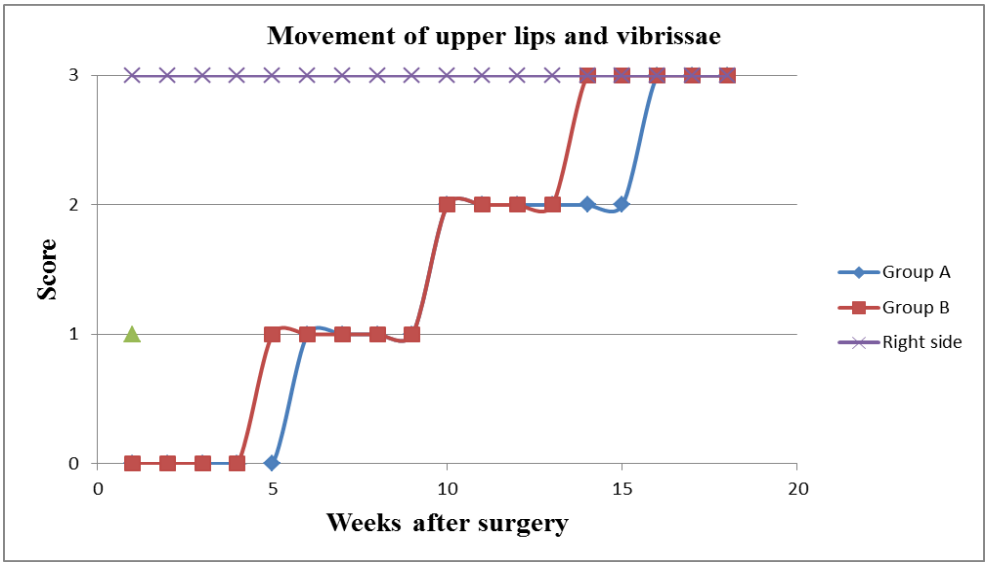

Fig. (1): Functional parameter of upper lips and vibrissae movement for left side and compared it with right normal side in all groups of surgery. The scales in groups A, and B, had significant difference ($p<0.05$) compared with right side.

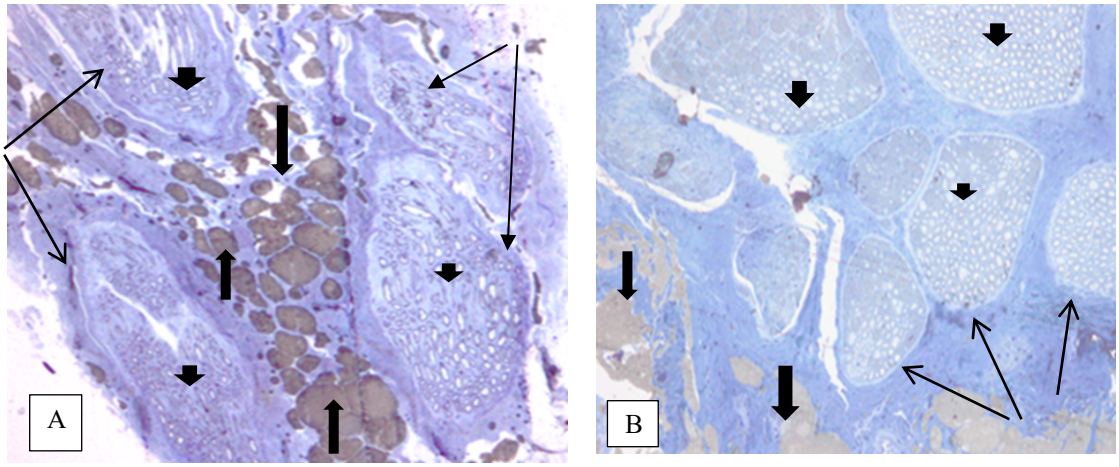

Fig. (2): Histological images of regenerated nerve section after toluidine blue stain X200 (**A)** in group A, show less organized regenerated myelinated axons (Arrow head), few nerve fascicles (Thin arrows) and adipose tissue infiltration between fascicles (Thick arrows). **(B)** In group B, show well organized regenerated myelinated axons (arrow head), much nerve fascicles (Thin arrow) and adherent adipose tissue (Thick arrow).

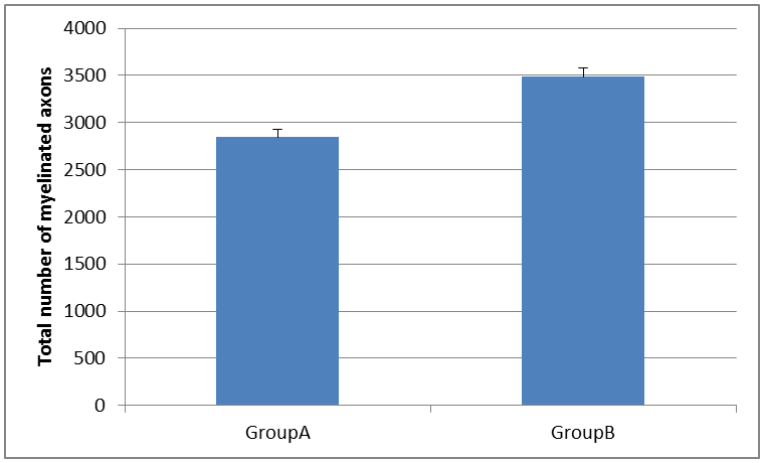

Fig. (3): Total number of myelinated axons at 120 days after surgery in group A and group B. The columns represent the mean ± SD (P <0.05).

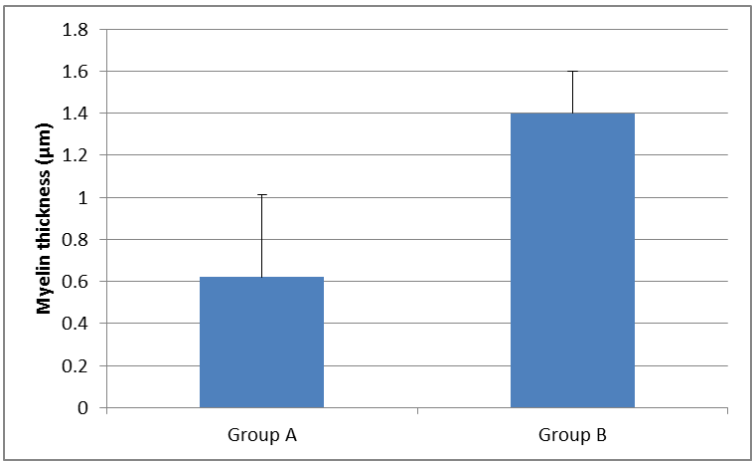

Fig. (4): Myelin thickness at 120 days after surgery in group A, group B. The columns represent the mean ± SD (P <0.05).

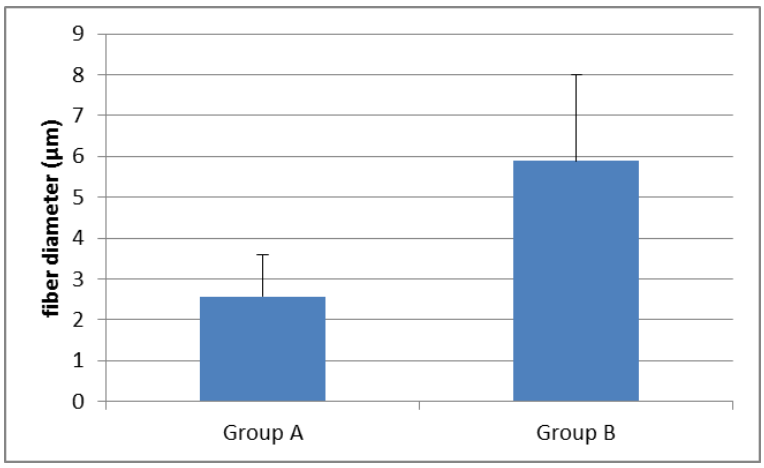

Fig. (5): Fiber diameter at 120 days after surgery in group A, group B. The columns represent the mean ± SD (P <0.05).

DISCUSSION

Adipose - derived stem cells (ADSCs) have the ability to promote regeneration and remyelination of damaged nerve axons and can be harvested less invasively with a higher yield, rapidly expanded and show low immunogenicity **(Faroni *et al.*, 2013).** Clinical evaluation of upper lip movement and attached vibrissae is often adopted in the facial nerve models as a functional test for facial nerve healing **(Yian *et al.*, 2001; de Faria *et al.*, 2006; Zhu *et al.*, 2015)**. The buccal branch of the facial nerve innervates the rabbit's *quadrates labii superioris* muscle, which plays a major role in movement of the upper lip and vibrissae **(Bowden and Mahran, 1960)**. The clinical improvement in upper lip movement and attached vibrissae was noticed earlier in group B. This explained the role of ADSCs in promoting the

functional recovery of the facial nerve. These results were in agreement with **(Watanabe *et al*., 2014)** in a rat facial nerve. ADSCs have angiogenic abilities leading to improved and accelerated vascularization and it also have anti-apoptotic effect leading to prevent neurons from dying and it produce numerous neurotropic growth factors such as nerve growth factor, which are essential for regeneration of nerve injuries **(Salgado *et al*.,2010; Lopatina *et al*.,2011and Kingham *et al*.,2014)**.The nerve regeneration across gap was better in group B than group A and this result may due to ability of ADSCs to promote growth and prevent neuronal death **(Zhao *et al*., 2009).** These results were coincident with **(Costa *et al*., 2013)** that improved the histological outcome of stem cells associated with PGA conduit in compared with empty PGA conduit for rat facial nerve defect regeneration. Morphometric analysis of the regenerated nerve sections was considered an objective method for evaluating the nerve regeneration and gives a perception of the quality of regenerated tissue **(Lewin *et al*., 1997 and Salomone *et al*., 2012)**. The increase in axon diameters and numbers in group B more than group A indicate beneficial effects of ADSCs in facial nerve regeneration which could lead to increase in the speed of nerve conduction. These results agree with **(Sun *et al*., 2011; Orbay *et al*., 2012 and Mohammadi *et al*., 2013)**, theirs demonstrated the beneficial effects of ADCS in the morphometric values of peripheral nervous repair. This study concluded that nerve reconstruction with PGA mesh seeded with adipose-derived stem cells improved the facial nerve regeneration in rabbit's more than empty PGA mesh.

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