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Full Length Research Paper

Fibrin sealant as scaffold can be a suitable substitute to autograft in short peripheral nerve defect in rats

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There is considerable evidence that peripheral nerves have the potential to regenerate in an appropriate microenvironment. In this study, the process of nerve regeneration through a fibrin scaffold was examined. 45 male Wistar rats were randomly divided into one control, Autograft (Auto) and two experimental groups, Epineurum (Epi) and Fibrin scaffold (Fib). Right sciatic nerve was exposed of which 5 mm was cut. The nerve defect was then bridged with a nerve autograft, empty epineurium and fibrin scaffold in the corresponding groups. All animals were examined one, three and five weeks after the operation to evaluate nerve regeneration and functional recovery employing light microscopy and walking track analysis, respectively. The gastrocnemius muscle contractility was also examined at 35th day post surgery in all groups using electromyography (EMG). Histological, functional evaluation and EMG evidences show that the nerve regeneration in both groups Auto and Fib were statistically equivalent and superior to that of Epi group ($P<0.01$). The present findings indicate that a fibrin scaffold enhances nerve regeneration as effective as a nerve autograft.

Key words: Sciatic nerve, fibrin scaffold, electromyography, autograft.

INTRODUCTION

The peripheral nervous system, in contrast to the central nervous system, has the ability to spontaneously regenerate injured axons (Jubran and Widenfalk, 2003). However, functional recovery is achieved upon complete axonal regeneration, which includes remyelination and reinnervation of the appropriate muscle and sensory targets (Millesi, 1984). After nerve crush, regeneration is usually successful, because the continuity of the endoneurial tubes is preserved. In contrast, there is limited axonal growth across gaps that result from complete nerve transection (Haastert et al., 2006). Nerve autografts are currently considered as the gold standard technique for the repair of peripheral nerve injuries; however, due to the disadvantages related to autograft (e.g., formation of neuroma, limitations in availability of donor site and donor site morbidity), interest to investigate other means of repairing peripheral nerve lesions has increased (Dellon and Mackinnon, 1988). Biomaterials such as veins, arteries, silicone, or polyglycolic acid allow relatively good nerve regeneration, especially in short nerve defects (Auba et al., 2006). Narakas (1988) revived the use of fibrin in nerve repair. Since then, its use has steadily gained popularity amongst the peripheral nerve surgeons. In the repair of

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rat median nerve, fibrin sealants produced less inflammatory response and fibrosis, better axonal regeneration, and better fiber alignment than the nerve repairs performed with microsuture alone. In addition, the fibrin sealant techniques were quicker and easier to use (Ornelas et al., 2006). Bozorg et al. (2005) have reported promising results with fibrin sealant in the repair of facial nerve in human beings.

Subsequent studies clarify the application of fibrin sealant as a glove but in this study the main aim was to examine the efficacy of fibrin sealant as scaffold in repair of cut rat sciatic nerve, as well as a comparison with the use of empty epineurium and nerve autograft.

MATERIALS AND METHODS

Animal grouping

The study was performed in 45 male Wistar rats, each weighing 200 to 250 g. The animals were randomly divided into three equal groups and underwent right sciatic nerve cut followed by repair using one of the following methods: Nerve autograft (group Auto), empty epineurium (Epi) and fibrin scaffold (Fib). In all animals, a five millimeter nerve gap was bridged at the time of surgery. The animals were then sacrificed one, three and five weeks after surgery for histological assessment. Therefore, groups of five animals underwent repair for 5 mm gap length at each time point. For surgical procedures and electrophysiological studies, the rats were anesthetized with a mixture of ketamine (60 mg/kg) and xylazine (10 mg/kg) given by intraperitoneal injection, and repeated as needed.

Preparation of fibrin scaffold

Fibrin albeit a filling material elsewhere, it serves as a scaffold in the present study due to its three dimensional (3D) structure.

Plasma was from human source. It is not necessary to remove plasma from cryoprecipitate and it should be dissolve into 10 ml of remaining supernatant plasma. Cryoprecipitate is a well defined blood product and is rich in fibrinogen. After addition of thrombin into the concentrated fibrinogen, fibrin would be formed. Concentration of fibrinogen could be adjusted to an optimum consistency. The fibrin that is formed from effect of thrombin on fibrinogen can be used as a biologic semi-solid scaffold and a few studies showed that it can support growth of cells. Prepared thrombin and fibrinogen were aliquoted and stocked. Whenever we needed them, we thawed and mixed them into a glass tube with 2 mm diameter therefore the consistency was the same.

The production of fibrin scaffold had four preparatory steps: Plasmaphoresis, cryo, fibrinogen and thrombin preparation.

Plasmaphoresis

About 230 ml of human plasma, obtained from donated blood from Iranian Blood Transfusion Organization was divided into three bags using plasmaphoresis. An anti-coagulant was added according to hematocrit level, from 1:12 to 1:8 prior to plasmaphoresis. The first bag received 50 ml of the end product and the second bag, received the rest. The bags were then frozen at -80°C and kept at -20°C for further analysis.

Cryo preparation

The second bag containing 180 ml of plasma was defrosted in a vertical position at 4°C for at least 12 h. The plasma was then centrifuged at 3500 rpm for 10 min. The supernatant was carefully and completely transferred to the third fresh bag using an extractor; the remaining 10 to 15 ml was left in the second bag to dissolve the yellow cryo precipitate.

Fibrinogen preparation

Enough protamin sulfates was added to the Falcon tube containing an equal amount of cryo. Then, the tube was centrifuged at 3000 rpm for 10 min. The supernatant was precisely and completely removed and enough 0.2 M sodium citrate was used to dissolve the precipitated fibrinogen.

Thrombin preparation

The first bag bearing fresh frozen plasma was defrosted at 37°C. Thrombin Processing Device (TPD) was turned on and kept running at standby position for 5 min at 45°C before thrombin was injected. Eleven milliliters of plasma was introduced into the TPD, we also made sure it was running at 90°C prior to injection. The device was turned upside down seven times to mix the plasma with kaolin and the powder of TPD. The TPD was then laid quiet in a horizontal position at laboratory temperature for 30 min. Subsequently, it was mixed for another five minutes and left in horizontal position again. Next, the solution was mixed and the device put in a vertical position. Finally, the thrombin-containing plasma was extracted.

To prepare fibrin scaffold during surgery, an equal amount of thrombin and fibrinogen was mixed, 1 ml each, to form a gel. Five minutes later, under aseptic condition, pieces of about 5 mm length × 2 mm thick of fibrin were then quickly obtained and added as scaffold to the sciatic gap already induced (Dresdale, 1985; Rock, 2007; Buschta, 2004; Weibrich, 2002; Zimmermann, 2003).

Surgical procedures

Once, the animals were acquainted with the animal house, after a 2-week stay, their right lateral thigh was shaved and the skin prepared with povidone iodone solution. The rat was placed in the prone position on a warming mat and under sterile condition a dorsolateral skin incision was made from the posterior thigh to the knee of the right hindleg and sciatic nerve exposed between the sciatic notch and the popliteal bifurcation by a gluteal muscle splitting incision. Under the operating microscope, after longitudinal incision in the epineurium, 5 mm of sciatic nerve below the sciatic notch (10 mm) was removed while epineurium was kept intact. In group Epi after cutting sciatic nerve, epineurium was sutured with 10-0 nylon suture. In group Fib, 5 mm sciatic gap was filled by fibrin scaffold (5 mm length and 2 mm diameter) and then epineurium was sutured with 10-0 nylon suture. In the group Auto, 5 mm of sciatic nerve distal to sciatic notch (10 mm) transected and then rotated for 180° before suturing it in place.

The skin wounds were closed with 4-0 nylon suture. After surgery, the rats were returned to individual cages for recovery and were permitted to mobilize freely with unlimited access to food and

Table 1. Electromyographic results of nerve repairs in different groups (Mean ± SD).

Group	EMAP (mV)	Latency (ms)
Auto	12.3 ± 3.2	1.44 ± 0.08
Epi	5.4 ± 0.89 [ab]	2.4 ± 0.55 [ab]
Fib	11.02 ± 3.6	1.5 ± 0.13

[a] denotes statistical significance P<0.01 compaired to Auto group;
[b] denotes statistical significance P<0.01 compaired to Fib group.

water.

Electromyographic assessment

To evaluate nerve regeneration, electromyographical measurements were performed 5 weeks after surgery in all three groups. The motor distal latency (DL) and the evoked muscle action potential (EMAP) of gastrocnemius muscle were measured using an electromyographic recorder (Biomed 3250). The sciatic nerve proximal to the site of cut was stimulated with an electric monophasic stimulus using needle electrodes. To reduce any possible interference, a ground electrode was placed inside the muscle adjacent to the nerve. The gastrocnemius response was recorded by cap electrodes placed on the gastrocnemius muscle. The distance between the site of stimulation and the muscle was 2 cm and this distance was kept constant in repeated studies.

Histomorphological study

One, three and five weeks postoperatively and after EMG assessment, the animals were sacrificed with an overdose of ketamine (200 mg/kg). Immediately after the operation site, the distal segment of sciatic nerve was harvested and fixed in a 2% glutaraldehyde solution at 4ºC for 12 h. Then, the nerve was dehydrated in increased concentrations of ethanol, passed through propylene oxide and embedded in epon resin and cross-sections (0.5 µm), distal to the neurorrhaphy site, using a microtome (Leica, Germany). All specimens were stained with 0.1% (w/v) toluidine blue in preparation for light microscopy. Morphometry was performed with an image analysis program (Image_ J, Ver 1.42, http://rsbweb.nih.gov/ij/download.html) (Abramoff et al., 2004). Video images were obtained with a digital camera (Nikon, Ds-Fil-L2, Japan) attached to a light microscope (Nikon, 50i, Japan). By using a modified version of Etho method, a manual count of myelinated fibers (MFs) was undertaken for five randomly selected square area (total = 0.02 mm^2) (Eto et al., 2003). These counts were then averaged to produce a mean estimate of myelinated fibers per one squared millimeter field.

Walking-track analysis

Walking-track analysis was performed 7, 21 and 35 days after the operation. After dipping the hindlimbs in ink, each rat was made walk down a 130×25 corridor freely. The foot prints were then analyzed to measure (1) distance from the heel to the third toe, called as the print length (PL); (2) distance from the first to the fifth toe, the toe spread (TS); and (3) distance from the second to the fourth toe, the intermediate toe spread (ITS) (De Medinaceli and Wyatt, 1982). All these measurements were taken from the experimental and normal sides. The measurements were used to calculate the factors as follows: Print length factor (1) (PLF) = (EPL-NPL)/NPL; (2) toe spread factor (TSF) = (ETS-NTS)/NTS; (3) intermediate toe spread factor (ITF) = (EIT-NIT)/NIT. These factors were then incorporated into Bain sciatic functional index-formula (Bain et al., 1989). SFI = -38.3 × PLF + 109.5 × TSF + 13.3 × ITF - 8.8. A SFI value of 0 is considered normal; a SFI of -100 was indicated to be total impairment, as it would be for a complete transaction of the sciatic nerve. If there is functional recovery due to regeneration techniques, the SFI should increase from -100 towards 0. So, SFI can be used as a parameter for measuring post-injury functional recovery.

Statistical analysis

In this study, all numerical data are presented as mean± standard deviation (Mean ± SD). Results were tested for statistical differences among the groups by using two-way ANOVA, followed by Tukey test as a post hoc test.

RESULTS

Electromyographic finding

By 35 days post repair, the EMAPs from the gastrocnemus muscle were reported in the groups of nerve repair with interposition autograft (Auto), empty epineurium (Epi) and fibrin scaffold (Fib). The difference between Fib and Auto groups with Epi group was significant (p<0.01), but no statistical difference was noted between Auto and Fib group. The results of the electromyographic studies of each group are shown in Table 1.

Histomorphometry finding

Regeneration of myelinated axons was observed in the distal segment of all groups. Examples of neural architecture are shown in Figure 2, distal to repair site for each group. The number of myelinated fibers (MFs) in all groups did not significantly differ one week after surgery. At 3 and 5 weeks post repair, histomorphometric analysis demonstrated a statistically significant differences (p>0.5) in number of MFs in nerve treated with the Fib and Auto groups compared to Epi group (P<0.01). There was no difference between the data of Auto and Fib group at these times (p>0.05). The average number of myelinated fibers in different treatment groups is shown in Figure 1.

Walking- track analysis

By the end of experiments, SFI values had increased in

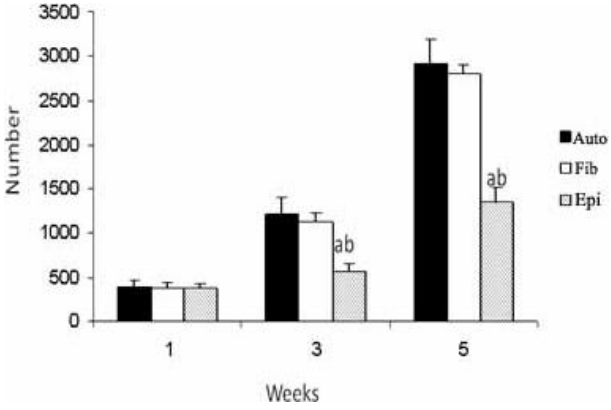

Figure 1. Number of MFs in Epineurum (Epi) and Fibrin scaffold (Fib) groups compared with Autograft (Auto) group. [a] denotes statistic al significance, P<0.01 compared to Auto group, and b denotes statistical significance P<0.01 compared to Fib group.

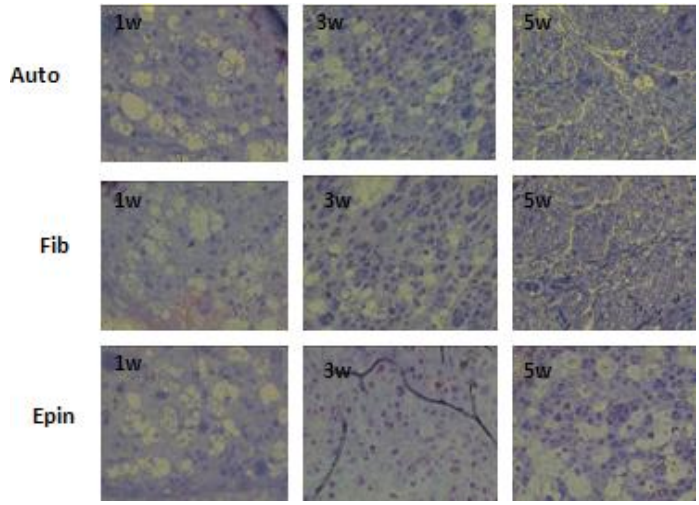

Figure 2. Photomicrographs of semithin sections at 1 week (1w), 3 (3w) and 5 weeks (5 w) post surgery in Auto., Fib. and Epi groups. Scale (bottom right) indicates of 50 µm (Toluidine blue staining, × 100).

Table 2. Results of walking track analysis in the three groups (Mean±SD).

	Weeks after surgery		
Group	**1**	**3**	**5**
Auto	-98.5±1.13	-85.2±3.46	-72.24±3.49
Epi	-98.1±1.1	-95.4±1.2 [ab]	-82.4±1.9 [ab]
Fib	-98.4±1.02	-88.6±5.4	-75.01±6.5

[a]denotes statistical significance, P<0.01 compared to Auto group. [b] denotes statistical significance P<0.01 compared to Fib group.

all groups, although not reaching the normal level. There was no significant difference in functional recovery among the three groups one week postsurgery; however, SFI value in Auto and Fib groups, as compared to Epi group significantly increased in weeks 3 and 5 after the operation (P<0.01). At the same weeks, there was no significant difference between SFI value in Auto and Fib group. The results of walking track analysis are presented in Table 2.

DISCUSSION

In this study, the effectiveness of the repair of 5 mm gap in the sciatic nerve with autograft, fibrin scaffold and empty epinurium was investigated. The results of this study showed that fibrin scaffold can repair gap peripheral nerve, as well as autograft. This finding is supported by the studies published by other researchers (Pittier, 2005; Galla, 2004; Martins, 2006). Previously fibrin was utilized as tissue glue to attach dissected nerve fiber end to end instead of suture (Ornelas, 2006; Jubran and Widenfalk, 2003). Therefore, it is not surprising why many reported researchers regarding fibrin scaffold in repair of gap peripheral nerve could not be found. Fibrin supports angiogenesis and tissue repair. Also naturally, it contains sites for cellular binding and has been shown to have excellent cell seeding effects and good tissue development (Amrani, 2001; Ye et al., 2000). Fibrin scaffold is a three-dimensional structure that can mimic the extra cellular molecules (ECM) to promote and guide actively the newly formed cells and tissues (Aper et al., 2004). Simon and colleagues demonstrated that EMAP four weeks after repair of a 4 mm gap in sciatic nerve, by direct microsurgical suture, significantly greater than repair with nerve autograft and collagen based nerve guide conduit (Simon et al., 2004). It seems that some properties of collagen, such as variability in cross-linking density and fiber size, unpredictable enzymatic degradation, possible side effects and mineralization (Lee et al., 2005) delay nerve regeneration, however no toxic degradation or inflammatory reactions were detected in the fibrin scaffold (Ye et al., 2000; Aper et al., 2004). These benefits can enhance nerve regeneration.

Bioactive signaling molecules, like growth factor, play a significant role in the cellular growth, proliferation and differentiation in the ECM *in vivo*. Fibrin scaffold allows incorporation of growth factor, bioactive peptides and proteins and thus can also function as a kind of delivery system for added biologically active substances such as vascular endothelial growth factor and basic fibroblast growth factor (Jeon et al., 2005; Bhang et al., 2007).

In conclusion, the data in this study demonstrate that

fibrin scaffold can be as proper as autograft both functionally and morphometrically and thus fibrin scaffold can be a suitable alternative for autogtaft.

REFERENCES

Abramoff MD, Magelhaes PJ, Ram SJ (2004). Image processing with Image. J. Biophoton. Int., 11: 36-42.

Amrani DL, Diorio Y, Delmotte (2001). Wound healing: Role of commercial fibrin sealants. Ann. NY. Acad. Sci., 936: 566–579.

Aper T, Teebken G, Steinhoff A (2004). Use of a fibrin preparation in the engineering of a vascular graft model. Eur. J. Vasc. Endovasc. Surg., 28: 296–302.

Auba C, Hontanilla B, Arcocha J (2006). Peripheral nerve regeneration through allograft compared with autografts in FK506-treated monkeys. J. Neurosurg., 105: 602-609.

Bain J, Mackinnon S, Hunter DA (1989). Functional evaluation of complete sciatic, peroneal and posterior tibial nerve lesions rat. Plast. Reconstr. Surg., 83: 129-132.

Bhang SH, Jeon O, Choi CY, Kwon YH, Kim BS (2007). Controlled release of nerve growth factor from fibrin gel. J. Biomed. Mater. Res. A., 80: 998–1002.

Bozorg GA, Mosnier I, Julien N, El Garem H, Bouccara D, Sterkers O (2005). Long term functional outcome in facial nerve graft by fibrin glue in the temporal bone and cerebellopontine angle. Eur. Arch. Otorhinolaryngol., 262: 404-7.

Buschta,C, Dettle M, Funovics PT (2004). Fibrin sealant produced by Cryoseal FS System:product chemistry, material properties and possible preparation in the autologous preoperative setting. Vox sanguinis, 86: 257-262

De Medinaceli L, Freed, WJ, Wyatt RJ (1982). An index of functional condition of rat sciatic nerve based on measurements made from walking tracks. Exp. Neural., 77: 634-643.

Dellon AL, Mackinnon SE (1988). An alternative to the classical nerve graft for the management of the short nerve gap. Plast. Reconstr. Surg., 82(5): 849–856.

Dresdale A (1985). Preparation of fibrin glue from single-donor fresh-frozen plasma. Surgery, 97: 750-754.

Eto M, Yoshikawa H, Fujimura H (2003). The role of CD36 in peripheral nerve remyelination after crush injury. Eur. J. Neurosci., 17: 2659-2666.

Galla TJ, Vedecnik SV, Halbgewachs J (2004). Fibrin/Schwann cell matrix in poly-epsilon-caprolactone conduits enhances guided nerve regeneration (an abstract). Int. J. Artif. Organs, 27(2): 127-136.

Haastert K, Christina M, Cordula M (2006). Autologous adult human Schwann cells genetically modified to provide alternative cellular transplants in peripheral nerve regeneration. J. Neurosurg., 104: 778-786.

Jeon O, Ryu SH, Chung JH, Kim BS (2005). Control of basic fibroblast growth factor release from fibrin gel with heparin and concentrations of fibrinogen and thrombin. J. Control Release, 105: 249–259.

Jubran M, Widenfalk J (2003). Repair of peripheral nerve transections with fibrin sealant containing neurotrophic factors. Exp. Neural., 181: 204-212.

Lee CH, Singla A, Lee Y (2005). Biomedical applications of collagen. Int. J. Pharm., 221: 1–22.

Martins RS, Siqueira MG, Ciro FS, Jose Pindaro PP (2006). Correlation between parameters of electrophysiological, histomorphometric and sciatic functional index evaluations after rat sciatic nerve repair. Arq. Neuropsiquiatr., 64(3-B): 750-756.

Millesi H (1984). Nerve grafting. Clin Plast Surg. 11: 105-113.

Narakas A (1988). The use of fibrin glue in repair of peripheral nerves. Orthop. Clin. North Am., 19: 187-198.

Ornelas L, Padilla L, Di Silvio M, Schalch P, Esperantes, Innfante RL, Bustamante JC, Avalos P, Varela D, Lopez M (2006). Fibrin glue: An alternative technique for nerve coaptation - PartII: Nerve regeneration and histomorphometric assessment. J. Reconstr. Microsurg., 22: 123-128.

Pittier RF, Sauthier JA, Hubbell H (2005). Hall, Neurite extension and *in vitro* myelination within three-dimensional modified fibrin matrices. J. Neurobiol., 63: 1–14.

Rock G, Neurath D, Semple E (2007). Preparation and characterization of human thrombin for use in thrombin glue. Transfusion Med., 17: 187-191.

Simon J, Christian K, Jeremy S (2004). A collagen-based nerve guide conduit for peripheral nerve repair: An electrophysiological study of nerve regeneration in rodents and non human primates. J. Comp. Neurol., 306(4): 685-696 .

Weibrich G, Kleis WK, Hafner G (2002). Growth factor levels in platelet-rich plasma and correlation with donor age, sex, and platelet count. J. Craniomaxillifac Surg., 30: 97-102.

Ye Q, Zund P, Benedikt S, Jockenhoevel SP, Hoerstrup S, Sakyama JA (2000). Fibrin gel as a three dimensional matrix in cardiovascular tissue engineering. Eur. J. Cardiothorac. Surg., 17: 587–591.

Zimmermann R, Arnold D, Strasser E (2003). Sample preparation technique and white cell content influence the detectable levels of growth factors in platelet concentrates. Vox Sang., 85: 2.