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A Novel Therapy to Promote Axonal Fusion In Human Digital Nerves

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Abstract

Background—Peripheral nerve injury can have a devastating impact on our military and veteran population. Current strategies for peripheral nerve repair include techniques such as nerve tubes, nerve grafts, tissue matrices, and nerve growth guides to enhance the number of regenerating axons. Even with such advanced techniques, it takes months to regain function. In animal models, polyethylene glycol (PEG) therapy has shown to improve both physiologic and behavioral outcomes after nerve transection by fusion of a portion of the proximal axons to the distal axon stumps. The objective of this study was to show the efficacy of PEG fusion in humans and to retrospectively compare PEG fusion to standard nerve repair.

Methods—Patients with traumatic lacerations involving digital nerves were treated with PEG after standard microsurgical neuroorrhaphy. Sensory assessment after injury was performed at 1 week, 2 weeks, 1 month, and 2 months using static two-point discrimination (2PD) and Semmes-Weinstein monofilament testing (SWM). The Medical Research Council Classification (MRCC) for Sensory Recovery-Scale was used to evaluate the level of injury. The PEG fusion group was compared to patient-matched controls whose data was retrospectively collected.

Results—Four PEG fusions were performed on four nerve transections in two patients. PEG therapy improves functional outcomes and speed of nerve recovery in clinical setting assessed by average MRCC score in week 1 (2.8 vs 1.0, $p=0.03$). At 4 weeks, MRCC remained superior in the PEG fusion group (3.8 vs 1.3, $p=0.01$). At 8 weeks, there was improvement in both groups with the PEG fusion cohort remaining statistically better (4.0 vs 1.7, $p=0.01$).

Conclusion—PEG fusion is a novel therapy for peripheral nerve repair with proven effectiveness in animal models. Clinical studies are still in early stages but have had encouraging results. PEG fusion is a potential revolutionary therapy in peripheral nerve repair but needs further investigation.

Study Type—Therapeutic Study

Level of Evidence—2

Keywords

Peripheral nerve injury; Polyethylene glycol; Axonal fusion; Nerve transection; Traumatic neuropathy

BACKGROUND

Peripheral nerve injury can have a devastating impact on our military and veteran population. Nerve recovery can take months or years as severed axons grow to target tissues from the injury site. Modern body armor and the widespread use of pre-hospital tourniquets have led to a dramatic increase in the number of wounded warriors with survivable injuries [1]. The unexpected consequence has been a dramatic increase in personnel with mutilated extremities.

Extremity amputations are debilitating injuries, and a review of the literature shows that extremity injuries make up 54% of combat wounds sustained in Operation Iraqi Freedom (OIF) and Operation Enduring Freedom (OEF) [2]. Moreover, the long-term function of a denervated, but salvaged hand is dismal. Interestingly, a recent review of service member injuries during OIF and OEF noted significant increases in brachial plexus injury, radial nerve injury, and ulnar nerve injury attributable to modern warfare.[3] The same study also noted that despite advanced medical treatment, there is still a significant jump in the incidence of amputation when comparing modern wartime to peacetime injuries. Taken together, these studies demonstrate that nerve injury is significant contributor to service member injury, disability, and limb loss. This provides a compelling rationale to develop improved nerve repair strategies.

Peripheral nerves are capable of regeneration but at a slow rate (1–2mm/day) with often poor functional recovery. Current advanced techniques of nerve repair using decellularized nerve allografts, tissue matrices and nerve growth guides rely on axonal outgrowth to denervated target tissues, which is often complicated by muscle atrophy [4,5]. The inability to rapidly restore the loss of function after axonal injury often results in poor clinical outcomes. Fusing the cut ends of axons back together could permit earlier nerve recovery and prevent Wallerian degeneration. The ability to fuse together severed axons and rapidly regain nerve function may dramatically alter functional outcomes for patients with mutilated extremity injuries and potentially even change the types of injuries for which limb salvage is attempted.

Additionally, the ability to fuse severed axons could potentially revolutionize limb replantation, reconstructive transplantation, and proximal nerve injury. A large barrier to

traumatic limb injury is nerve injury. Direct nerve repair, when possible, is the gold standard in these types of injuries. However, when nerve repair is not possible, surgeons employ other techniques, which include autografting, nerve conduits, and nerve transfers [6,7]. Despite these efforts, outcomes remain dismal, and the capability of axonal fusion could positively impact outcomes.

Polyethylene glycol (PEG) has been investigated as an agent to promote axonal fusion in animal models. For decades, polyethylene glycol (PEG) has been used to fuse cells in order to immortalize desired cell lines such as monoclonal antibody producing B-cells [8]. PEG facilitates lipid bilayer fusion by removing water from the lipid bilayer at the damage site, decreasing the activation energy required for plasmalemmal leaflets to fuse [9,10]. PEG mediated rapid recovery in severed halves of invertebrate giant axons has been measured by intra-axonal dye diffusion studies and action potential conduction through the lesion site in vitro [11]. Using PEG, morphological and physiological continuity of severed or crushed mammalian sciatic and spinal axons have been rapidly restored in vitro, ex-vivo, and in vivo [12–14]. PEG fusion has also been shown to enhance functional recovery in rat models [15].

Given the repeated success of PEG fusion in animal models, human trials are the next logical step. We present a case series of the first human PEG fusions. The objective of this study was to show the efficacy of PEG fusion in humans and to retrospectively compare PEG fusion to standard nerve repair. PEG fusion is a novel technique with the potential to revolutionize nerve repair.

MATERIALS and METHODS

All procedures in human subjects were performed according to the ethical standards of Thammasat University, Pathumthani, Thailand, where the operations were performed. Approval was obtained from the Vanderbilt University Institutional Review Board to retrospectively review standard nerve repairs to serve as the control group. A Vanderbilt surgeon (W.T.) was a participating surgeon in training for the procedures performed in Thailand. Follow up was conducted in Thailand by a combination of Vanderbilt and Thammasat University surgeons. The control group was selected from patients treated by the Department of Plastic Surgery at Vanderbilt.

Inclusion Criteria

The inclusion criteria for our study consisted of sharp nerve injuries requiring intervention that were operated on within 12 hours of injury. Exclusion criteria were avulsion injuries and nerve injuries that occurred more than 12 hours before the operative repair. Patients were offered the opportunity to participate in the study, and informed consent was required.

Surgical Procedure

Nerve sites were prepared for repair with wound debridement and irrigation. Nerve endings were dissected in preparation for primary repair. After resection of nerve endings to a fresh edge, the nerve ends were irrigated with a calcium free solution (Plasma-Lyte A, Baxter, Deerfield, IL) for two minutes. Nerves were primarily repaired using standard microsurgical technique with a 9–0 nylon suture. The neuroorrhaphy site was treated with 1% methylene

blue (Faulding, Aguadilla, Puerto Rico) for one minute (Figure 1a), followed by two minutes of PEG 3350 kD 50% W/V in water application to promote fusion of axonal stumps (Figure 1b). The closely apposed axolemmal ends were irrigated with calcium containing solution (Lactated Ringer, Hospira, Lakeforest, IL) for another two minutes. Surgical wounds were closed with case appropriate dressing and splinting.

Post-operative follow up

Post-operative follow-up was conducted in Thailand by a combination of surgeons from Vanderbilt and Thammasat University. Sensory recovery assessment of the digital nerves was performed at 1 week, 2 weeks, 1 month, 2 months, and 3 months using static two-point discrimination (2PD) and Semmes-Weinstein monofilament testing (SWM). The Medical Research Council Classification (MRCC) (SDC 1) for Sensory Recovery-Scale was used to evaluate the level of injury. The Michigan Hand Questionnaire and Short-Form 12 Health Survey were used to assess the patients' postoperative outcomes.

Static two-point discrimination—Two-point discrimination is a standard method of evaluating functional sensibility in the hand. The normal range for thinner areas of the skin (finger pulp) is 2 to 4 millimeters and for thicker skin is up to 6 millimeters. A standard two-point discrimination measurement tool was used in our patients to measure their sensation.

Semmes Weinstein Test—The Semmes Weinstein test is a discriminative test using monofilaments to identify threshold stimulus necessary for perception of touch sensation. Using standard monofilaments of different sizes (2.83, 3.61, 4.31, 4.56, 6.65) (SDC 2), the patients were tested by placing the filaments at a 90-degree angle against the skin. The patients were tested on their ability to feel the various sizes of filaments.

Michigan Hand Questionnaire—The Michigan Hand Questionnaire (MHQ) is a hand-specific outcomes instrument that measures the outcomes of patients with hand or wrist injuries. The MHQ has six distinct scales: overall hand function, activities of daily living, pain, work performance, aesthetics, and patient satisfaction with hand function. The MHQ has 37 core questions, and higher scores indicate better hand performance. Scores are on a scale of 1–100.

Short Form-12 Health Survey—The Short Form-12 Health Survey is a generic assessment of health-related quality of life. The survey contains 12 questions that are combined, scored, and weighted to produce two scores: the physical and mental health composite scores (PCS and MCS). Scores are on a scale of 0 to 100.

Control group

We retrospectively collected data on similar patients treated at Vanderbilt for similar injuries. Patients were demographically matched to our patients from Thailand based off age, gender, and similarity of injury and repair. Inclusion criteria included patients who underwent sharp nerve transections with primary repair. Operative notes and post-operative notes were reviewed to collect information on nerve repair and post-operative sensory recovery. Two

point discrimination, Semmes-Weinstein, and MRCC data were collected from post-operative follow-up visits.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 5 for Mac OS X (GraphPad Software, San Diego, CA). MRCC scores were compared weeks 1, 4, and 8 using Mann Whitney U-Test, a non-parametric test. MRCC score S0-S2 was categorized as group 1, S3 (meaningful recovery) as group 2, S3+ as group 3 and S4 as group 4. Probability of type I error of less than 5% ($p < 0.05$) was used to determine statistical significance.

RESULTS

Four PEG fusions were performed on four nerve transections in two patients. In this group, there was 1 male (age 16) and 1 female (age 17). All four nerve injuries were sharp complete transections. All nerve injuries were repaired within 12 hours of injury. No complications were observed in the PEG group.

The first patient had a sharp digital nerve transection at 45mm from the tip of her left index finger. She underwent a successful digital nerve repair with PEG fusion within 12 hours from initial injury. At 3 weeks, she had gross sensation to touch with a static two-point discrimination of 12mm and a Semmes Weinstein test of 4.56. At 5 weeks, her two-point discrimination was 9mm, and Semmes Weinstein test was 4.56. At two months, she had returned to work part-time with no pain and minimal numbness and tingling, and her two-point discrimination was 5mm, and Semmes Weinstein Test was 4.31. The MRCC score for this patient was S3+. (Table 1, Figure 2) At 120 days postoperatively, the patient's MHQ score was 93 (scale 1–100), SF-12 PCS score was 56.6 (scale 0–100), and SF-12 MCS score was 60.8 (scale 0–100).

The second patient had had three digital nerve injuries at 45, 55 and 55 mm from the tip on his left index, middle and little fingers. He underwent three successful digital nerve repairs with PEG fusion within 12 hours from initial injury. At 1 week, he had gross sensation to touch with a two-point discrimination of 10 mm in the left index finger, 10 mm in the left middle finger, and 12 mm in the left little finger with a Semmes Weinstein test of 4.31 for all fingers. At two weeks, his two-point discrimination improved to 7 mm in the left index finger, 6 mm in the left middle finger, and 8 mm in the left little finger while the Semmes Weinstein test remained stable at 4.31 in all fingers. At one month, his two-point discrimination improved to 5 mm in the left index finger, 6 mm in the left middle finger, and 4 mm in the left little finger while the Semmes Weinstein test remained stable at 4.31 in all fingers. At two months, his two-point discrimination improved to 4 mm in the left index finger, 5 mm in the left middle finger, and 4 mm in the left little finger while the Semmes Weinstein test remained stable at 4.31 in all fingers. At three months, his two-point discrimination remained stable at 4 mm in the left index finger but improved to 4 mm in the left middle finger and 3 mm in the left little finger while the Semmes Weinstein test improved to 3.61 in all fingers. The MRCC score in this patient was S3+. (Table 1 and Figure 2) At 180 days postoperatively, the patient's MHQ score was 93 (scale 1–100), SF-12 PCS score was 53.1 (scale 0–100), and SF-12 MCS score was 62.6 (scale 0–100)..

We compared our PEG-treated patients from Thailand with patient matched controls from Vanderbilt. Our control group had 6 patients (five males, one female). The average age was 27.2 years. All nerve injuries were sharp transections similar to our experimental group, which also underwent primary repair. The average MRCC score at 1 week was 1.0 (Standard Deviation=0), 4 weeks was 1.3 (SD=0.82), and 8 weeks was 1.7 (SD=0.55). No complications of repair were observed in our control group.

PEG therapy improves functional outcomes and speed of nerve recovery in clinical setting assessed by average MRCC score in week 1 (2.8 vs 1.0, $p=0.03$). At 4 weeks, MRCC remained superior in the PEG fusion group (3.8 vs 1.3, $p=0.01$). At 8 weeks, there was improvement in both groups with the PEG fusion cohort remaining statistically better (4.0 vs 1.7, $p=0.01$). (Figure 3, SDC 3)

DISCUSSION

Peripheral nerve injury is a large challenge for surgeons, and our cases series describes a novel therapy for peripheral nerve repair. Current strategies for nerve repair rely upon axonal outgrowth, which is ~ 1–2mm/day, but Wallerian degeneration, which begins within 1–2 days after injury [16], can limit recovery potential. In proximal nerve injuries, irreversible muscle atrophy often occurs before axonal outgrowth reaches the target tissues. Our case series highlights PEG fusion as a potential new strategy for nerve repair, which has been extensively tested in animal models and is in its early stages in clinical testing.

PEG fusion is a novel therapy that fuses cut axons, which helps eliminate the need for axonal outgrowth for nerve recovery. The first artificially induced axonal fusions were performed using PEG to repair cut crayfish axons [17] and other invertebrate axons [18–21]. Our lab and a collaborating lab [17, 22–26] subsequently PEG-fused transected mammalian axons to rapidly produce morphological and functional axonal continuity. The mechanism of PEG fusion relies upon maintenance of a calcium-free environment during which an antioxidant methylene blue and PEG are applied. Methylene blue is an anti-oxidant that prevents the endogenous sealing mechanisms in cut nerve endings [26]. The inhibition of sealing maintains nerve endings in a state receptive to axonal fusion. When PEG is introduced, it artificially induces closely apposed membranes of severed axonal ends to flow into each other. PEG removes water from the area allowing the lipid bilayers of apposed axon stumps to fuse together. This produces a partial repair of plasmalemmal membranes that are then perfused with Ca^{2+} -containing saline to promote vesicles to accumulate and seal remaining holes at the lesion site. Figure 4

Our lab and a collaborating lab have demonstrated morphological and functional axonal continuity after PEG fusion with direct nerve end-to-end repair, nerve allografts, and nerve autografts [15, 22, 26]. Axonal continuity was demonstrated in these studies via electrophysiology studies. More recently, the Institute of Imaging Sciences at our institution has utilized diffusion tensor imaging (DTI), a magnetic resonance imaging (MRI) technique that is an emerging diagnostic tool in nerve injury, to demonstrate the success of PEG fusion. Figure 5 demonstrates representative tractography of porcine sciatic nerves that were

repaired using PEG fusion. MRI tractography is a new and developing technology, which provides further evidence of PEG axonal fusion.

In our current study, PEG fusion of digital nerve injury was performed and revealed improved nerve recovery and functional outcome as early as one week. This was evident from the return of sensation to touch by two-point discrimination and Semmes Weinstein Monofilament testing with the MRCC score of S3+ in our patients. Unfortunately, there are fewer methods of evaluation of nerve repair in a clinical setting compared to animal models. The physical exam remains the gold standard for follow up of nerve injury, and from our experience with PEG fusion in a clinical setting, this nerve fusion technique appears to be translatable to humans. This success is exciting as it represents the first evidence of salvage of distal axons after complete nerve transection in humans.

In our series, only digital nerves were treated with PEG fusion. While patients historically do not recover normal functional sensibility after digital nerve injury, patient satisfaction after repair is high [27]. However, patients are at risk for worse outcomes and decreased quality of life with more proximal nerve injuries [28]. If PEG fusion is effective for proximal nerve injuries, it may help prevent muscle atrophy and loss of innervation of distal muscles, which routinely occurs in proximal nerve injury. However, more trials with different types of nerve injuries are needed.

PEG fusion is a technique that can be rapidly and easily adopted. PEG has a variety of applications in research and medicine, as it is cheap and readily available. It also has low toxicity, which should allow for fewer side effects. Our current technique for PEG fusion is to apply PEG over the neurorrhaphy site by intraoperative injection. This could easily be adopted into current nerve repair practice. However, to ensure uniform PEG delivery to axonal endings, our lab is currently investigating techniques to deliver PEG optimally and uniformly to the nerve repair site.

PEG fusion has the potential to make a great impact on traumatic nerve surgery in both a military and civilian setting. Our investigation of PEG fusion in both animals and humans has only included immediate repair of sharp transections of nerves. In most clinical settings, nerve injuries are not repaired immediately after injury, and nerve injuries are not always sharp transections. In our case series, we only used PEG fusion on patients who had sharp nerve transections and had a recent nerve injury. Moving forward, we plan to investigate the optimal timing of PEG fusion after nerve injury and whether a critical window exists, and we also will extend our investigation into different type of nerve injuries such as avulsion and crush. Despite the limitations of PEG fusion to sharp nerve transections, we believe PEG fusion is still applicable to all nerve injuries given there is evidence PEG fusion is effective in the use of autografts and allografts. Nerve injuries are often complicated by gaps requiring interpositional grafts. Our initial investigation in complicated nerve injuries in animal studies is promising, but more rigorous study in human trials is necessary.

Our animal studies have involved PEG fusion of the sciatic nerve, but the aforementioned cases involve repair of distal sensory nerves. PEG fusion is not motor or sensory specific, but achieving motor and sensory nerve alignment may play a role in success of PEG fusion.

Complications or limited function may occur due to the misalignment of sensory-motor nerve fibers with fusion technique in mixed nerves. The complications from misalignment may be improved by fascicular based neuroorrhaphy, and additional microsutures may be required to improve alignment and minimize the effects of nerve rotation. More studies are needed to assess the effectiveness of nerve repair techniques in PEG fusion.

Our current human investigation in PEG fusion presented here has limitations. While PEG fusion shows promise in our animal studies and current cases series, more rigorous investigation is needed. Our case series was not a randomized control trial, and it is also limited by its small size. Due to the fact we did not have a concurrent control group with our PEG group, we recognize that no definitive conclusions about the superiority of PEG fusion in human nerve repair can be drawn at this time. However, our limited post-operative follow-up revealed successful rapid functional recovery from the PEG fusion, but long-term follow-up would allow us to fully understand the effects of PEG fusion. Our group is currently conducting a randomized control clinical trial of PEG fusion, which should allow us to develop a further understanding of this novel technique in humans. Given the long history of the study of PEG fusion in animal studies and our preliminary human reports presented here, there is reason to be optimistic about the future of PEG fusion nerve repair in humans.

Despite being in an early stage of investigation, PEG fusion shows great potential. Our lab has demonstrated repeated and reliable success in animal nerve repair via different modalities. Our experience with PEG fusion in human nerve repair is encouraging and deserves further exploration. As we move forward with our clinical investigation of PEG fusion, we plan to determine optimal delivery methods in addition to examining long-term outcomes. Peripheral nerve repair is in its infantile stages, and PEG fusion represents a potential large step forward.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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A

B

Figure 1:

(a) Human digital nerve repair after application of 1% methylene blue. (b) Human digital nerve repair with PEG application

SDC 1: Medical Research Council Classification (MRCC) score for sensory assessments: Scale to determine the level of sensory function of a target area in the hand

SDC 2: Semmes Weinstein Test

Figure 2:
Two-point discrimination of PEG fusion patients over time

Figure 3:

The PEG therapy group demonstrated statistically significant functional recovery at 1 week, 4 weeks and 8 weeks post-operatively (P value<0.05).

SDC 3: Mann-Whitney U Test for Statistical Analysis of MRCC score for PEG vs No PEG groups

Figure 4:
Mechanism of PEG axonal fusion. In the absence of PEG, calcium-mediated vesicles form and seal axonal endings, preventing fusion. PEG and MB reduce vesicle formations and promote axonal fusion. (Reprinted from Rodriguez-Feo et. al 2013)

Figure 5:

Diffusion tensor tractography of porcine sciatic nerves. (A) Representative tractography of transected nerves harvested immediately following repair. No cut (left) (n=4), Cut and Repair (middle) (n=4), Cut and Repair with PEG (right) (n=4). (B) Fractional Anisotropy (FA; mean $\pm$ standard deviation) was assessed at three sites: 5mm proximal; repair site, and 5mm distal to the repair site. PEG treated nerves were associated with a statistically significant increase in FA at the site of injury compared to cut + repair nerves ($p < 0.05$)

Table 1:

Sensory testing of two patients after PEG fusion

Patient	Age (yr)	Finger	Distance	Sensory testing	1 wk	2 wk	3 wk	4 wk	5 wk	8 wk	12 wk
1st Pt	18	LIF	45 mm	2PD (mm)			12		9	5	
				SWM			4.56		4.56	4.31	
2nd Pt	16	LIF	55 mm	2PD (mm)	10	7		5		4	4
		LMF	55 mm		10	6		6		5	4
		LLF	45 mm		12	8		4		4	3
		LIF	55 mm	SWM	4.31	4.31		4.31		4.31	3.61
		LMF	55 mm		4.31	4.31		4.31		4.31	3.61
		LLF	45 mm		4.31	4.31		4.31		4.31	3.61