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Peripheral Nerve

Fibrin Glue Acutely Blocks Distal Muscle Contraction after Confirmed Polyethylene Glycol Nerve Fusion: An Animal Study

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Background: Polyethylene glycol (PEG) is a synthetic, biodegradable, and hyperosmotic material promising in the treatment of acute peripheral nerve injuries. Our team set out to investigate the impact of fibrin glue upon PEG fusion in a rat model.
Methods: Eighteen rats underwent sciatic nerve transection and PEG fusion. Electrophysiologic testing was performed to measure nerve function and distal muscle twitch. Fibrin glue was applied and testing repeated. Due to preliminary findings, fibrin glue was applied to an uncut nerve in five rodents and testing was conducted before and after glue application. Mann-Whitney U tests were used to compare median values between outcome measures. A Shapiro-Wilk test was used to determine normality of data for each comparison, significance set at a P value less than 0.05.
Results: PEG fusion was confirmed in 13 nerves with no significant change in amplitude ($P = 0.054$), latency ($P = 0.114$), or conduction velocity ($P = 0.114$). Stimulation of nerves following PEG fusion produced distal muscle contraction in 100% of nerves. Following application of fibrin glue, there was a significant reduction in latency ($P = 0.023$), amplitude ($P < 0.001$), and conduction velocity ($P = 0.023$). Stimulation of the nerve after application of fibrin glue did not produce distal muscle twitch. Five uncut nerves with fibrin glue application blocked distal muscle contraction following stimulation.
Conclusions: Our data suggest that fibrin glue alters the nerve's function. The immediate confirmation of PEG fusion via distal muscle twitch is blocked with application fibrin glue in this experimental model. Survival and functional outcome studies are necessary to understand if this has implications on the long-term functional outcomes. *(Plast Reconstr Surg Glob Open 2024; 12:e5535; doi: 10.1097/GOX.0000000000005535; Published online 19 January 2024.)*

INTRODUCTION

Polyethylene glycol (PEG) is a synthetic, hyperosmotic compound capable of fusing the lipid bilayer membrane of severed nerves following approximation.[1] Following structural nerve approximation and leaching calcium, the application of PEG can combat Wallerian degeneration, providing immediate nerve function, increased recovery, and preservation of neuromuscular junctions.[1,2] Current research on PEG-treated nerve repairs has shown promising results with recovery rates more than 80% in animal models, with upwards of 100% of axons repaired following fusion.[3,4] PEG does not provide structural support to nerve endings, despite fusing the axonal plasma membrane.[5,6] A clear benefit to the use of PEG is that nerve function can be both observed and tested immediately following successful axonal fusion.[1,3] Successful nerve function immediately after repair can be evaluated via electrophysiological recordings of amplitude and nerve conduction velocity, and by visualizing distal muscle force generation via direct nerve stimulation.[1,4,5] This provides surgeons and researchers with the ability to assess nerve fusion efficacy in real time.

The use of fibrin glue for nerve coaptation has been studied in animals since the 1940s with clinical trials

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starting in the 1970s.[7] There have been several studies comparing its efficacy in nerve coaptation against suture repair.[8,9] Many nerve surgeons utilize fibrin glue for its ease of use, reduced scarring to the nerve endings, and comparable outcome measures.[10,11]

To our knowledge, no study has investigated the use of fibrin glue alongside PEG to treat nerve injuries. With growing support for fibrin glue nerve repairs in the literature[7–11] and increased interest in the use of PEG to study nerves immediately after injury, our team evaluated nerve function immediately following repairs with PEG and fibrin glue. We sought to determine if the two can be used together.

Takeaways

Question: Is PEG safe to use when fibrin glue is employed for neurorrhaphy?

Findings: Fibrin glue alters axonal electro-conduction, preventing distal muscle twitch and the ability to instantly confirm PEG nerve fusion.

Meaning: If PEG is utilized for nerve injury repair, fibrin glue would prevent immediate confirmation of successful fusion, long term survival studies would be needed to assess compatibility.

MATERIALS AND METHODS

This is a nonsurvival rat sciatic nerve model electrophysiological study approved by our Institutional Animal Care & Use Committee and in accordance with humane treatment of research animals to assess the immediate impact of fibrin glue on PEG nerve fusion.

Surgical Technique

Eighteen male Lewis retired breeder rats underwent an acclimation period of 2 weeks. Following acclimatization, aseptic surgery using standard microsurgical techniques under both loupe and microscope magnification (Leica WILD M690) was performed. The sciatic nerve was exposed in the thigh. Baseline sciatic nerve conduction velocities and signal amplitudes were measured from the intact nerve. Next, the nerve was transected in the midportion of the thigh above the nerve trifurcation using microsurgical scissors.

An immediate primary neurorrhaphy utilizing PEG nerve fusion was then performed following described techniques. The nerve endings were irrigated with 0.5-mL calcium-free solution (PlasmaLyte-A), followed by 0.1 mL methylene blue (Acros Organics) antioxidant. The nerve ends were approximated using 9-0 suture (Ethicon) under microscopic magnification. The area was then treated with 0.5 mL PEG (5% weight, 20 kDa PEG in phosphate-buffered saline) for 90 seconds to achieve axolemma fusion before irrigation with Lactated Ringer's solution. PEG fusion was confirmed by stimulating the sciatic nerve proximal to the PEG fusion with a physiologic impulse (0.1 mA) and observing a contraction of the tibialis anterior muscle. If a larger, supraphysiologic impulse (1.0 mA and higher) was necessary to induce distal muscle contraction, then the PEG fusion was categorized as unsuccessful. An intraoperative photograph through the microscope of a sciatic nerve after neurorrhaphy and PEG fusion is shown in Figure 1.

Successful PEG fusion in 13 subjects was then followed by circumferential application of fibrin glue (Tisseel; Baxter) at the neurorrhaphy site. Electrophysiology testing and stimulation was then repeated.

Based on a modification to our original Institutional Animal Care & Use Committee–approved protocol, we also assessed the immediate impact of the fibrin glue in five normal rat sciatic nerves with similar electrophysiologic testing. In these five rats, we simply applied the fibrin glue circumferentially to the intact sciatic nerve. The same electrophysiology testing as was done in the PEG-repaired nerve was performed on the intact sciatic nerve before and after fibrin glue application.

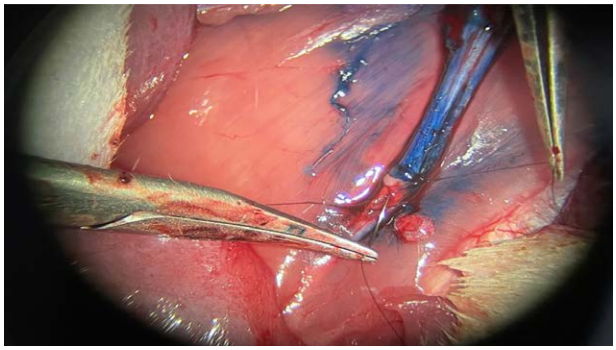

Fig. 1. Intraoperative photograph through the microscope of a rat sciatic nerve following transection, suture repair, and PEG fusion. The nerve is stained blue following the addition of methylene blue, an agent for antioxidation during the PEG fusion process.

Electrophysiology

Electrophysiologic testing was performed with an electromyography and nerve conduction stimulator machine (Cadwell Sierra) to record nerve conduction velocity, nerve latency, and action potential amplitude. A single pulse of 0.15 mA was applied to the sciatic nerve for a duration of 0.05 milliseconds to induce nerve conduction. Latencies were measured in 0.0167-millisecond intervals. This current was conducted across a fixed 1.2-cm gap to maintain consistency within electrophysiologic testing. To study distal muscle twitch, a bipolar stimulator and microhook electrode was used to deliver an electrical impulse of 0.1 mA both proximal and distal to the site of repair. If nerve conduction was present, this direct current stimulation would activate the rodent tibialis anterior muscle and cause visible ankle flexion.

Statistical Analysis

Mann-Whitney U tests were used to compare median values between primary outcome measures. A Shapiro-Wilk test was used to determine normality of data for each comparison. Statistical significance was set at a P value less than 0.05.

RESULTS

A PEG fusion was performed and confirmed in 13 rodents in our study. Fibrin glue was applied to an intact sciatic nerve in the final five rodents after preliminary findings in the first 13.

Impact of PEG Fusion on an Uncut Nerve

Compared with an uncut nerve baseline, electrophysiology measurements suggest that nerves with a PEG fusion demonstrate no significant change in amplitude (435 versus 278 μV, $P = 0.054$), latency (0.45 versus 0.38 milliseconds, $P = 0.114$), or conduction velocity (31.1 versus 36.8 m/s, $P = 0.114$) (Table 1). A visible distal muscle twitch was observable in all 13 PEG fusions.

Impact of Fibrin Glue after Successful PEG Fusion

Compared with an uncut nerve baseline electrophysiologic testing, application of fibrin glue reinforcement of a PEG fusion significantly reduced latency (0.45 versus 0.36 milliseconds, $P = 0.023$) and amplitude (435 versus 163 μV, $P < 0.001$), and there was a significant increase in conduction velocity (31.1 versus 38.9 m/s, $P = 0.023$) (Table 1). When comparing these results to a nerve after PEG fusion without fibrin glue, the application of fibrin glue significantly decreased amplitude (299 versus 150 μV, $P = 0.019$), but there was no significant difference in latency or conduction velocity (Table 1). After fibrin glue was applied to the otherwise successful PEG fusion, no distal muscle twitch was visible with the same physiologic, proximal nerve stimulation (Fig. 2).

Impact of Fibrin Glue on a Normal Nerve

Compared with baseline measures of uncut nerves, application of fibrin glue circumferentially to an intact nerve significantly reduced latency (0.40 versus 0.34 milliseconds, $P = 0.034$), increased conduction velocity (35.0 versus 41.2 m/s, $P = 0.034$), and resulted in an insignificant reduction in amplitude (352 versus 248 μV, $P = 0.076$) (Table 1). After fibrin glue was applied to the otherwise normal sciatic nerve, no distal muscle twitch was visible with the same physiologic, proximal nerve stimulation (Fig. 2).

DISCUSSION

Application of fibrin glue to successful PEG fusion blocked immediate visible, muscle contraction distal to the fusion in this experimental model. Muscle contraction seems to be the most widely used immediate confirmation of successful fusion available to the surgeon, this loss of visible feedback of an otherwise successful fusion was a

Table 1. Median Electrophysiology Readings for PEG Fusion and Fibrin Glue

	Amplitude (μV)	*P*	Latency (ms)	*P*	Conduction Velocity (m/s)	*P*
PEG conduction velocity not normally distributed ($P = 0.023$)						
Uncut nerve	434.50	0.054	0.45	0.114	31.12	0.114
PEG + sutures	277.90		0.38		36.80*	
Uncut nerve amplitude not normally distributed ($P = 0.002$)						
Uncut nerve	352.00*	0.076	0.40	**0.034**	35.04	**0.034**
Uncut nerve + fibrin glue	248.20		0.34		41.23	
PEG conduction velocity not normally distributed ($P = 0.015$)						
PEG	298.60	**0.019**	0.39	0.418	36.08*	0.454
PEG + fibrin glue	150.10		0.36		38.89	
Uncut nerve	434.50	**<0.001**	0.45	**0.023**	31.13	**0.023**
PEG + fibrin glue	163.20		0.36		38.89	

Values in boldface are statistically significant.
Mann-Whitney U tests were performed.
Statistical Significance ($P < 0.05$).
*Shapiro-Wilk distributions.

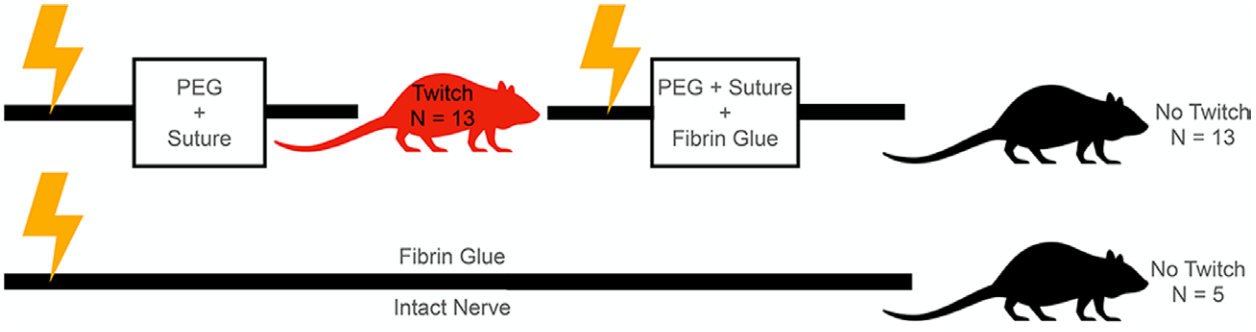

Fig. 2. Thirteen animals underwent nerve transection, and a PEG fusion was performed using suture coaptation. PEG fusion was successfully achieved in all 13 subjects. Stimulation produced 100% muscle twitch with 0.1 mA of stimulation proximal to the site of nerve fusion. Application of fibrin glue prevented muscle activation with stimulation. In an additional subset of five healthy sciatic nerves, fibrin glue prevented muscle twitch with direct stimulation.

significant finding as we try to understand the role of fibrin glue in PEG nerve fusion. Typically, after neurorrhaphy without PEG fusion, a nerve is not tested either before or after application of fibrin glue. However, with the advent of PEG, intraoperative confirmation of successful fusion appears essential. This small study suggests that application of the fibrin glue significantly alters nerve function immediately. Although the long-term effects of fibrin glue on PEG fusion remain unknown, there does appear to be an immediate electrophysiologic impact. In addition to blocking a visible muscle contraction, the application of the fibrin glue to the PEG fusion also impacted the velocity and amplitude of the conducted signal based upon our recordings.

Application of the fibrin glue to the normal sciatic nerve in the smaller group of five rats affirmed our observations related to the immediate impact of the fibrin glue on nerve electrophysiology. We believe that the impact on the nerve is independent of the PEG fusion. Visible downstream twitch was lost, the amplitude was diminished, and the velocity increased. These findings, in both the PEG fusion and the intact nerve, occurred when the fibrin glue was applied circumferentially. Small gaps in the circumferential application of the fibrin glue allowed for observed distal muscle twitch. Although the number of nerves studied in this report is relatively small, because we were observing 100% blockage of twitch, there seemed to be little value in studying a larger cohort. Rather, survival data seem to be the next logical step.

Despite a large body of literature investigating the use of fibrin glue in both human and animal nerve repair, we have not discovered any studies in the literature investigating immediate nerve electrophysiology and/or function following repair with fibrin glue.[6,12] Before PEG technology, immediate testing was not particularly relevant to clinicians. Based on our findings, we observed that fibrin glue, when applied circumferentially around a nerve, impedes, or blocks nerve conduction by reducing amplitude below muscle conduction threshold. This is evidenced by lack of distal muscle contraction when directly stimulating the nerve proximal to the repair in any of our experimental arms. We hypothesize that this effect is attributable to fibrin glue having a lower electrical resistance than the nerve itself. This would account for the mildly decreased amplitude (352 versus 248 μV, $P = 0.076$) and increased conduction velocity (35.0 versus 41.2 m/s, $P = 0.034$) observed when applying fibrin glue to an intact nerve. This effect was also seen when testing nerves with confirmed PEG fusion with suture repair and then repeat nerve testing after the application of fibrin glue (amplitude 299 versus 150 μV, $P = 0.019$, 100% distal muscle twitch before fibrin glue, 0% distal muscle twitch after application). This was an unexpected finding, which has not been previously reported.

Most fibrin glues utilized in nerve surgery are dissolved in the human body by 2 weeks, which also accounts for the reported increased incidence of repair dehiscence.[7,10] Because the glue is theoretically completely dissolved before electrophysiologic testing is performed in animal survival studies (typically at 4 wk), this effect of conduction blockade would not be observed in the reported literature investigating fibrin glue and nerve repair.[9,11]

Based on these findings, we cannot definitively conclude that PEG and fibrin glue are incompatible together. Survival studies with long-term electrophysiologic testing would be necessary to conclude if they can be combined for nerve repair. The intrinsic electroconductive properties of fibrin glue prevent immediate or intraoperative confirmation of PEG fusion, which eliminates a considerable advantage of its use in clinical settings and may thwart its benefits in the future of PEG fusion nerve treatment.

CONCLUSIONS

PEG has shown significant and promising experimental results in the treatment of peripheral nerve injuries and may become a mainstay of acute nerve injury treatment in humans. There is strong evidence to support the use of fibrin glue in direct nerve repair; however, it is unclear if these two modalities can be utilized in conjunction. This study demonstrates that fibrin glue prevents immediate nerve function tests via a hypothesized reduction in electrical resistance as evidenced by inability to produce distal muscle contraction with direct stimulation, increased conduction velocity, and decreased amplitude. Although the long-term effects of fibrin glue on PEG fusion remain unknown, there is an immediate impact on the PEG fusion, preventing the ability to immediately assess nerve fusion.

This information is important for nerve surgeons who will assess in vivo downstream muscle twitch after PEG application to confirm fusion. Survival studies are necessary to determine if fibrin glue inhibition is simply a temporary electrophysiologic phenomenon, or if fibrin glue may impact PEG fusion outcomes adversely over time.

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DISCLOSURES

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