# Ethyl-cyanoacrylate is acutely nontoxic and provides sufficient bond strength for anastomosis of peripheral nerves

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**Abstract:** Anastomosis is a common technique for the union of severed nerve trunks. This is commonly performed with sutures, a process that can be both time consuming and injurious to tissue. One promising alternative to suturing is the use of adhesives to join the severed segments. Cyanoacrylate-based glues have been used clinically as a surgical adhesive for soft tissues. However, the acute effects of these glues on nerve electrophysiology and the tensile strength of the rejoined tissues have not been evaluated. Using a guinea pig model, we analyzed the mechanical properties of transected sciatic nerves repaired with epineural application of ethyl-cyanoacrylate and the

# INTRODUCTION

Peripheral nerve injury is a common occurrence in the United States and clinical case reports suggest as many as 20% of nerve trauma victims suffer complete nerve laceration.<sup>1</sup> A common technique in the treatment of acute injury is the surgical anastomosis of transected peripheral nerves using sutures. Although sutures are the clinical gold standard for coapting nerves, they also present some difficulties, including the increased procedural time associated with their application and the potential for inducing additional damage at the suture line.<sup>2</sup> The use of adhesives to join the damaged nerves thus represents an attractive alternative to sutures. Adhesives that have been investigated for use in neural anastomosis include fibrin glues and cyanoacrylates.<sup>3,4</sup> Cyanoacrylates are especially attractive due to their mechanical strength and fast cure times.<sup>5</sup> While

short term consequences of cyanoacrylate application on impulse conduction. Results showed that nerves coapted with ethyl-cyanoacrylate were capable of bearing *in vivo* forces. Additionally, no acute effects on conduction were observed in uninjured sciatic nerves exposed to ethyl-cyanoacrylate. In conjunction with long term *in vivo* reports from literature, the current results support the use of cyanoacrylates in nerve repair. © 2008 Wiley Periodicals, Inc. J Biomed Mater Res 90A: 750–754, 2009

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some studies have investigated the chronic tissue reaction and bonding of cyanoacrylates,<sup>4,6</sup> the short-term consequences of cyanoacrylates on nerve tissue have not been thoroughly evaluated. Therefore, the present study attempts to address the acute effects of ethyl- cyanoacrylate (CA) on the functional and structural properties of repaired nerves. The bonding strength was assessed by tensile testing of severed guinea pig sciatic nerve anastomosed with CA. Moreover, the acute functional effects of CA exposure on nerve tissue were measured with *ex vivo* electrophysiology.

## MATERIALS AND METHODS

#### Nerve isolation

The Purdue Animal Care and Usage Committee approved all experimental protocols for the procurement, handling, and disposal of animals or animal tissues. Adult female guinea pigs (325–450 g) were anesthetized by intramuscular injection of ketamine (80 mg/kg), and xylazine (12 mg/kg), and acepromazine (0.6 mg/kg). After perfusion with oxygenated Kreb's buffer solution at 15°C, the

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sciatic nerves were carefully isolated and removed. Following nerve excision, the extraneous connective tissue and muscle were removed using a no. 15 scalpel. Extreme care was taken when isolating and handling the nerves to minimize tissue damage and samples were moistened periodically to prevent dessication. Nerves were stored in Kreb's solution at 4°C prior to mechanical testing. For electrophysiological recordings, the sciatic nerves were split into the tibial and peroneal components. To aid recovery from the surgical process, nerves were stored in oxygenated Kreb's buffer (1–4 h) before commencement of electrophysiology.

#### **Tensile testing**

Prior to mechanical testing, rectangular rubber grips (Henkel Consumer Adhesives) were attached to the sciatic nerves using Super Glue (ethyl-cyanoacrylate, Loctite). This step was necessary to prevent the nerve slippage during stretch. Half of the specimens used were intact while the other half were transected with surgical scissors. The severed nerve group was repaired with a thin coat of CA. Care was taken to ensure the nerves were joined in an end-to-end anastomosis (Fig. 1). Experimental samples were allowed to cure for five minutes to ensure adequate bonding. Samples were moistened regularly with Kreb's solution to prevent dehydration.

Prior to stretch, the dimensions of the nerve samples were measured and tabulated using calipers to ensure an unstressed reference length. Samples were loaded into a Test Resources<sup>®</sup> 100Q250 computer controlled mechanical testing system (TestResources). The system was zeroed to the unstressed reference length of the nerve sample. Nerves were then stretched to failure at a deformation rate of 10 mm/min. For all samples, position and load were recorded 64 Hz. Data was exported through WinCom<sup>®</sup> (ADMET) and analyzed on a PC. To obtain an accurate cross-sectional area for data analysis, histological sections of the sciatic nerves of comparably sized guinea pigs (n = 4) were obtained and analyzed via Image Pro.

# Data analysis

Engineering strain was calculated as the change in length divided by the reference length. Ultimate strain refers to the distension at which mechanical failure occurred. Burst strength was the force recorded by the load cell at failure. The Young's modulus was approximated by dividing the estimated stress by the measured strain. The work to failure was calculated by numerical integration of the area under the load-strain curve and gives a value for the work preceding failure.

# Electrophysiological recording

A modified sucrose gap chamber was used to assess sciatic nerve impulse conduction function.<sup>7</sup> The endpoints of our functional assessment included compound action potentials (CAP) and latency. The CAP is defined as the sum of all evoked action potentials, with both motor and sensory



**Figure 1.** Mechanical testing of repaired nerve. A: Schematic of the CA application and tensile testing. A small drop of glue was placed on the epineurium of anastomosed nerves. Rubber pads were used to clamp the nerves to the tensile testing apparatus. B: Photograph of tensile testing device with nerve loaded. [Color figure can be viewed in the online issue, which is available at www. interscience.wiley.com.]

pathways recorded. The acquired CAP waveforms also gave latency (time from stimulus to CAP peak) data. Briefly, nerve samples (n = 7) were placed in the sucrose gap chamber, properly sealed and allowed to come to electrical equilibrium. When CA was applied, the fluid level was lowered and a small quantity of CA was placed on the central nerve segment and allowed to cure. After curing, fluid levels were returned to normal and electrophysiological recording reestablished. Recordings were made using a bridge amplifier (Neurodata Instruments) and data analysis performed using custom Labview software (National Instruments) on a PC as previously described.<sup>7</sup> Instantaneous waveforms and time histories were obtained. The nerves were maintained at physiologic temperatures. Results from CAP and latency were normalized to the preglue controls.

#### Statistical analysis

All data are reported in the form of mean  $\pm$  standard deviation. A student's *t*-test was used to determine differences in means. A *p*-value of less than 0.05 was considered to be statistically significant.

# RESULTS

# Mechanical properties of intact nerve

Quasistatic tensile tests of the intact nerve showed typical viscoelastic behavior (Fig. 2). Initially, little force was incurred within the intact tissue. This phase was followed by a linear trend, until nerve failure occurred. The mechanical properties are shown in Table I. Structural failure occurred at an average strain of  $0.25 \pm 0.04$ . Stress values were more difficult to obtain, as measuring the cross-sectional area of the nerve was complicated by excessive tissue compliance. Accordingly, an average cross-sectional area of 1.135  $\pm$  0.234 mm<sup>2</sup> was obtained from histological sections of comparably sized animals. Subsequent modulus computations were estimated from this value and the linear portion of the elongation plots.

# Mechanical properties of CA repaired nerve

Mechanical properties for the CA repaired samples are shown in Table I. The reapposed nerves were able to sustain on average, about 30% of the



**Figure 2.** Elongation curves of guinea pig sciatic nerve. (A) Intact nerve force-elongation plots show typical viscoelastic response. Inset: measured mechanical properties included strain to failure ( $S_F$ ), load to failure ( $L_F$ ) and work done (W). (B) CA repaired nerves demonstrate much lower failure loads and strains prior to failure. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE I Mechanical Properties of Sciatic Nerve

	Intact Nerve $(n = 7)$	CA Repaired Nerve $(n = 8)$
Burst strength (N) Ultimate strain Ultimate stress (MPa) <sup>b</sup> Young's modulus (MPa) <sup>b</sup> Work to failure (mJ)	$\begin{array}{r} 3.11 \pm 0.72 \\ 0.25 \pm 0.04 \\ 2.74 \pm 0.63 \\ 15.50 \pm 4.16 \\ 8.76 \pm 2.33 \end{array}$	$\begin{array}{c} 0.93  \pm  0.28^{a} \\ 0.17  \pm  0.049^{a} \\ 0.82  \pm  0.25^{a} \\ 7.07  \pm  0.74^{a} \\ 1.96  \pm  1.14^{a} \end{array}$

<sup>a</sup>Denotes statistical significance between the CA repaired and intact nerves (p < 0.05).

<sup>b</sup>Indicates values calculated from average cross-sectional areas.

intact nerve load. The strain to failure was also lower, with an average value of  $0.17 \pm 0.05$ . The estimated Young's modulus of the repaired nerve was  $7.07 \pm 0.74$  MPa, compared to  $15.50 \pm 4.16$  MPa for intact nerve. This finding suggests that the glue/ tissue interface may have been weak or the glue was not fully hardened. The work required for bond failure was  $1.96 \pm 1.14$  mJ. In all cases, the failure occurred at the glue joint. However, some curves show a double failure, where one side of the CA joint debonded but the nerves were still partly joined.

# Nerve electrophysiology

The electrophysiogical data showed ethyl-cyanoacrylate to be nontoxic in terms of the short-term conduction response. Ten minutes after the application of the glue, no statistically significant changes in the nerve CAP magnitude or latency were observed when compared with the preglue phase (Fig 3). Similarly, the instantaneous CAP waveforms retained their distinctive shape. Note that during glue application/curing, the electrophysiology could not be accurately obtained since the media volumes had to be reduced below the electrode level for glue application. When the nerve was removed from the recording chamber, solidified CA could be seen surrounding the nerve.

#### DISCUSSION

We currently show that direct apposition of severed nerve tissue is possible when a small quantity of ethyl-cyanoacrylate is applied to the epineural sheath. The mechanical testing of the repaired nerves suggests CA to be an acceptable compound for imparting strength and preserving nerve elasticity. The glue anastomosed nerve retained approximately 30% of the intact nerve load and approx 23% of the intact nerve work to failure. Although the cyano-



**Figure 3.** *Ex vivo* electrophysiological data. Representative time histories of compound action potential amplitude (A) and latency (B) before and after application of CA glue. (C) Statistical comparisons of the average normalized ratios show no statistical significance between the CAP or latencies pre- and post-glue (at 10 min post glue). Note that during CA application, the electrophysiology could not be reliably obtained. [Color figure can be viewed in the online issue, which is available at www.interscience. wiley.com.]

acrylate bond fails under lower loads than intact nerve, the cyanoacrylate bond strength appears to be sufficient to withstand subpathological in vivo forces. Our lab has shown that irreversible functional deficits occur at 5–10% supraphysiological stretch,<sup>8</sup> while cyanoacrylate samples stretched to 17%. This finding is further corroborated by prior work in vivo demonstrating long-term structural stability of cya-noacrylates.<sup>9,10</sup> Suturing, the current clinical standard for nerve anastomosis, has been shown to fail at loads of 0.61  $\pm$  0.22 N with rat sciatic nerves and in the range of 1-2 N with human cadaveric digital nerves.<sup>11,12</sup> These values are not significantly superior to the CA repaired data (0.93  $\pm$  0.28 N) and we therefore conclude that CA is an appropriate adhesive for maintaining nerve anastomosis in both acute and chronic timeframes.

The widespread use of CA has been limited due to reports of toxicity. Some higher homologous forms of CA have been suggested to be histotoxic,<sup>13</sup> and earlier studies report high tissue reactivity, inflammatory response and contraction in nerve diameter.<sup>3</sup> On the other hand, later experiments have shown that when used as a substitute for sutures, CA provides adequate long-term stability, minimal tissue reactivity and does not inhibit axonal regeneration.<sup>10</sup> These discrepancies may be attributed to choice of CA, differences in application technique and quantity of glue applied. If seepage of glue occurs at the suture line, the CA can cause intense tissue reaction, with the foreign body response tending to push the glue fragments inward.<sup>13</sup> In the current report, the glue was thinly applied to the epineurium, thereby minimizing seepage into the nerve core.

The current short-term electrophysiological data show ethyl-cyanoacrylate does not affect CAP or latency 10 min after application. In this period, the CA cured and may be considered to be functionally inert. We presume that the CA film did not penetrate the connective tissue layers of the peroneal/tibial nerves and that direct axon exposure to CA was minimal. In conclusion, the current findings show CA to be tolerable in short-term electrophysiological experiments and that long term toxicity is unlikely. In previous investigations, we have shown that membrane fusogens can restore action potentials in severed nerve fibers.<sup>14</sup> This data, in conjunction with the current findings, yields promise for CA and membrane fusogens in PNS repair techniques.

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