Artificial nerve graft using glycolide trimethylene carbonate as a nerve conduit filled with collagen compared to sutured autograft in a rat model

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Abstract—A study was conducted to compare the regeneration of rat peroneal nerves across 0.5 cm gaps repaired with artificial nerve grafts (ANG) versus sutured autografts (SAG). The ANG model is composed of a synthetic biodegradable passive conduit made of glycolide trimethylene carbonate (GTMC) filled with a collagen matrix (predominately Type I collagen, derived from calf skin, and with the telopeptide ends left intact). Axonal regeneration was studied in 11 long-term animals (two at 6 months and nine at 9 months). The nerves were studied by qualitative and quantitative histological, electrophysiological, and functional assays. Axonal regeneration with the ANG was equal to SAGs as measured by axonal diameters, physiological, and functional methods, although the SAG demonstrated statistically higher axonal counts.

Key words: *artificial nerve graft, autograft, axonal regeneration, biomaterials, collagen matrix, traumatic nerve injury.*

INTRODUCTION

In some traumatic nerve injuries, nerve loss results in a significant gap between the cut ends of the nerve that cannot be closed by an end-to-end repair (1,2,3,4). The present treatment of choice is the use of an autograft to bridge the gap (3,4). The major limitation of the autograft

is the requirement of a donor nerve. Homografts and heterografts have been evaluated as alternatives to autografts, but have been found to be immunologically unacceptable (5,6). Although it may be possible to suppress the immune system during regeneration through a homograft (7), one must weigh this with the risks of immunosuppression. Therefore, the development of an artificial nerve graft (ANG) is one method to solve both problems of availability and rejection by the immune system. Alternatives to the autografts that have been tried include homografts, heterografts, and artificial substitutes for nerve grafts (1,3,6,8,9). Recently, clinical studies of artificial nerve grafts have included polyglycolic acid (PGA) tubes (7) and veins (10), either implanted empty and subsequently filled with autologous serum, or filled before implantation with autologous blood products.

Several groups have been experimentally attempting to develop an ideal medium for axonal regeneration (11,12,13,14). Various macromolecules, including laminin, fibronectin, heparan sulfate, and collagen, have been analyzed and shown to have properties important for regeneration (11,15,16,17,18,19,20). Our current interest lies in the role of collagen on axonal regeneration in the artificial nerve graft. Important advantages of using collagen over other biomaterials include the relative ability to manipulate its biochemistry and microstructure (21).

We have previously studied Zyderm® Collagen Implant (ZCI) (composed of Type I with some Type III collagen)

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as an extracellular matrix for nerve regeneration (22) because of its hypoantigenicity and history of use *in vivo* (23). This collagen has been treated to remove the telopeptide ends from the triple-helix to reduce its antigenicity (24). Previous studies by us and others (25) have shown that nerves regenerate through this matrix; however, regeneration was shown to be inferior compared to sutured autografts (SAG) (22). This may result from the manufacturing process producing particles or clumps of collagen fibers up to 250 μm in diameter (24). These particles probably have a higher concentration than the average of 3.5 percent collagen. The final structure of Zyderm may render this collagen less than ideal as a cellular regeneration matrix.

In this study, we therefore chose to use a collagen with a structure more suitable for nerve regeneration. This Type I collagen was used in a gel form which results in a loosely woven matrix that provides spaces for regeneration; although this collagen retains its telopeptide ends, the overall antigenicity of collagen is low. The telopeptides play an important role in collagen fiber reassembly (21). This affects the precipitation kinetics, microstructure, and behavior of the collagen in multiple ways that may be advantageous for a nerve regeneration extracellular matrix.

The evaluated artificial nerve grafts were composed of resorbable tubes filled with a growth medium composed of collagen as a possible alternative to an autograft. Instead of PGA tubes, tubes of glycolide trimethylene carbonate (GTMC) were used because it is less reactive, possesses a controllable resorption time, and has better handling characteristics than PGA. The resorption time of GTMC can be made greater than that of PGA by adding methylene groups, making the structure more hydrophobic and more resistant to hydrolysis. In addition, the methylene moiety makes GTMC more flexible, unlike PGA which is brittle; therefore, GTMC is less likely to fragment when the grafts are flexed by the movement of the animal. Finally, GTMC is pliable enough to receive sutures, which facilitates its use surgically.

The interest in finding an ideal medium for axonal growth and passive devices for bridging gaps resulting from resected nerves has spanned over a century (5,8). These passive devices have included a wide range of biological and nonbiological materials (8). Early artificial nerve grafts provided a tube filled with blood or some plasma product to span the nerve gap (26). In a few cases, only threads were used to span the gap with no surrounding tube (27). The early attempts were uniformly unsuccessful, both experimentally and clinically.

In 1944, Weiss reviewed prior studies of artificial nerve grafts (5). His experiments demonstrated that tubes filled with blood with or without threads as guides would successfully act as artificial nerve grafts (6). Experiments using autogenous veins as passive conduits for nerve regeneration found them to be acceptable, but far from being ideal artificial nerve grafts (10,28). Workers using pseudosynovial or mesothelial tubes have reported moderate success (11,16,29,30,31,32), while others experimenting with various synthetic resorbable materials also had moderate success (7,33). Although in some of these studies autologous blood products filled the conduits, none of these studies were conducted to determine the most appropriate matrix material with which to fill the tube as a growth medium for axonal regeneration.

The natural extracellular matrix is composed of multiple proteins, including laminin, fibronectin, and collagen (21). The extracellular matrix exerts a strong influence over cellular regeneration, and in particular, nerve regeneration (34,35,36,37). Cell activity is regulated by contact with the collagenous extracellular matrix, not only for purposes of morphogenesis, but also for maintenance and adaptation of the differentiated state (38,39). There is ample evidence that cells can physically respond to the orientation of the substrate on which they rest (40,41,42,43).

Although only a part of the extracellular matrix, collagen is endowed with many unique properties. Cells respond to the chemical nature of collagen by interacting with attachment sites. They also respond to the porosity (cross-linking affects this porosity, along with strength, stiffness, and persistence *in vivo*) of the collagen, its antigenicity, its orientation, its mobilization of enzymes and other substances (44,45). These parameters can be manipulated to potentially yield enhanced nerve regeneration. Collagen is only mildly immunoreactive (21), due in part to masking of potential antigenic determinants by the helical structure, so that further decreasing antigenicity may not be all that important for our purposes. Finally, recent experiments have shown that silicone tubes filled with collagen resulted in successful bridging of nerve gaps (46).

There may be a number of reasons why collagen in the form of Zyderm may not be ideal for nerve regeneration. The physical form of Zyderm apparently tends to restrict migration of cells to narrow channels, at least until the dense particles or blocks have been degraded (23). Cell penetration must occur around these dense blocks through areas of least resistance (i.e., largely along planes of low fiber density). A sufficiently high collagen concentration, even if entirely homogenous, impedes cellular immigra-

tion in general (21). This is also probably true for nerve regeneration and may explain our limited success in our first artificial nerve graft experiments using Zyderm.

Our second study yielded more favorable results (47). We constructed an ANG composed of a synthetic biodegradable passive conduit made of PGA filled with a collagen extracellular matrix (predominately Type I collagen, derived from calf skin, and with the telopeptide ends left intact). This ANG was used in the repair of 0.5 cm gaps in the rat peroneal nerve. Statistical analysis yielded equal results between the sutured autograft and our ANG as measured by qualitative and quantitative histology, physiology, and functional (toe spread) analysis.

After finding a matrix which would support axonal regeneration, we sought out a conduit material which would be more applicable to our needs. Although PGA was effective as a biomaterial for the repair of short gaps, longer gaps would necessitate the use of biomaterials with different characteristics. The biomaterial would need to be less reactive, possess a controllable resorption time, and have better handling characteristics than PGA. Our search yielded GTMC. The resorption time of GTMC can be made greater than that of PGA by manipulating its structure (GTMC: Davis and Geck, Pearl River, NY). GTMC conduits can maintain their tube-like structure for upwards of 3 months (unpublished data).^{*} In addition, GTMC is flexible, unlike PGA which is brittle; therefore, GTMC is less likely to fragment when the grafts are flexed by the movement of the animal. GTMC is pliable enough to receive sutures which facilitates its use surgically. We are currently working on creating a porous GTMC tube, which other investigators have shown plays a significant role in nerve repair using tubes.

MATERIALS AND METHODS

Eleven Sprague-Dawley white male rats were used to compare artificial nerve grafts to sutured autografts (Figure 1). All animals survived the postoperative period.

Adult rats weighing approximately 250 grams each were deeply anesthetized with intraperitoneal sodium pentobarbital 5 mg/100 gm. The peroneal nerve was used as a test site. The peroneal nerve is a single fascicle (0.6 mm diameter) which arises from the bifurcation of the sciatic nerve. All surgical dissections and repairs were done using an operative microscope with magnification of 10 to 25 $\times$.

Figure 1.

Drawing of the rat model showing the surgical repair of the peroneal nerves with the artificial nerve graft (ANG) and sutured autograft (SAG). The SAG is on the left, and the ANG is on the right.

In each animal, both peroneal nerves were exposed and a 0.5 cm-long segment was removed from the distal portion of each nerve 5 mm distal to the sciatic bifurcation. One randomly selected side in each animal was repaired using an autograft from the opposite peroneal nerve excised segment. This was sutured using 10-0 nylon on a straight 70 micrometer diameter needle, requiring two sutures per end of nerve. The sutures were placed through the perineurium only.

The other side in each animal was repaired using an artificial nerve graft (Figure 2). The artificial nerve grafts were composed of GTMC tubes slightly larger in diameter than the nerves, 10 mm long, and filled with a liquid collagen (Collagen [C3511]: Sigma, St. Louis, MO) that subsequently gelled in the tube. The tube was split longitudinally and the nerve was pulled into the tube both proximally and distally for a distance of 2.5 mm. A blood clot was then allowed to form around both ends of the GTMC tube to hold the two nerve stumps secure within the tube without the need for sutures. Eleven animals were evaluated after long-term regeneration from 6 to 9 months by qualitative and quantitative histology, physiology, and functional assays.

Qualitative histology provided an assessment of the organization of the proximal and distal repair site. It also provided an assessment of reaction to the surgical materials (nylon suture, GTMC tubes, and the collagen within the tubes). The qualitative histological evaluations were used only as guides to the events which occurred at the

^{*} Personal communication. Conversation with R.F. Vallentini, 1990.

Figure 2.

Drawing of the artificial nerve graft model. The top drawing represents the glycolide trimethylene carbonate (GTMC) tube with the collagen bridge filling the 5 mm gap. The middle drawing represents the proximal and distal ends of the transected nerve. The lower two drawings show the two ends of the nerve being placed with the GTMC tube.

repair site. The organization of the repair site was ranked on a 5-point scale from fail to poor to fair to good to excellent. The reaction was ranked using a scale ranging from no reaction, to minimal reaction, to moderate reaction, to extreme reaction (48). Because of the presence of sutures in the SAG specimens, and occasional remnants of GTMC in the ANG specimens, it was not possible to do the evaluation in a completely blind method. All histologic evaluations were done by one histologist to avoid inter-tester bias.

Nerve repair site biopsies were fixed in 3 percent cacodylate buffered glutaraldehyde for a minimum of 24 hours. The site of repair was embedded in paraffin. Longitudinal sections were cut serially at 6 to 8 micrometers. Half of the sections were stained with Bodian stain (silver stain) to determine the organization of the regenerated axons. The following subjective qualitative assessments were given: excellent (normal axonal alignment); good (70 percent to 99 percent alignment); fair (40 percent to 69 percent alignment); poor (10 percent to 39 percent align-

ment); and, failure (no axonal alignment). The other half of the sections were stained with standard hematoxylin-eosin stain to evaluate tissue inflammatory reaction. This evaluation assigned the following subjective qualitative assessments: no reaction (0 WBC/HPF*), minimal (0-50 WBC/HPF*), moderate (50-100 WBC/HPF*), and extreme (>100 WBC/HPF*).

Quantitative histology was used to determine the axon diameter histogram (Figure 3) and the average diameter of the myelinated axon population just distal to the graft site (48). The average diameter provides a measure of axonal regeneration which is difficult to determine from axon counts because of branching (49). Nerve biopsies 5 mm distal to the repair site were taken at the termination of the study. The biopsies were fixed in cacodylate buffered 3 percent glutaraldehyde for 24 hours, then in 1 percent osmium tetroxide for 1 hour. The fixed biopsies were embedded in Epon and cut into 1 micrometer sections with an ultramicrotome, using a glass knife. They then were mounted on a microscope slide and stained with phenylene diamine. These sections were used to determine the count and diameter of regenerated myelinated axons.

Axonal cross-sectional area determination and counts were accomplished using a Macintosh® II computer system linked to a DEST Scanner. Each black and white photomicrograph (400 ×) of sections distal to the repair

Figure 3.

Diagram of the preparation of the fiber diameter histogram (FDH). (1) Cross-section is taken just distal to graft and a histological slide is prepared; (2) the histological section is photographed and the axons are manually traced; and, (3) a computer system is used to convert the traced axons into histograms.

Figure 4.

Diagram of the electrophysiological study. The neuroscope triggers the stimulator to stimulate the nerve either just proximal or just distal to the repair site. The resultant signal is recorded at the most proximal site available (i.e., as the nerve exits the spinal cord at the sciatic notch) and sent to the neuroscope for analysis. The proximal nerve is crushed to yield a monophasic signal.

represented approximately 15 percent of the cross-sectional area of the peroneal nerve. Three photomicrographs of each nerve section were used for this purpose. Tracings of these photographs exclusive of unmyelinated axons and blood vessels were digitized and a computer program then determined the area within the myelinated axons from an algorithm. The diameter was then determined from this area. The axonal count was subsequently determined by summing the number of axons in the percentage of nerve counted and calculating the total number for the whole nerve.

Quantitative physiology was used to measure the population of regenerated axons. The compound action potential was used to physiologically evaluate axon regeneration across the repair site *in vivo* (Figure 4) (50). The area under the monophasic compound action potential was determined by electronically computing the integrated mean compound action potential (IMCAP) (51). The area under

the monophasic compound action potential provides a measure of the proportion (population) of myelinated axons with distal connections (49), that is, regenerated axons.

As in the original nerve repair, the test animals were given appropriate anesthesia, and all surgery was performed under magnification. The repair site was exposed surgically, and the area was bathed in mineral oil maintained at 35 degrees Centigrade. Two sites were used to stimulate the nerve, one just distal to the repair site, and one 5 mm proximal. These sites were dissected free of connective tissue and placed on platinum wire stimulating electrodes approximately 12 mm apart, without disturbing the repair site (Figure 4). A baseline monophasic recording of the compound action potential (CAP) was obtained by severing and crushing the nerve at the sciatic notch (just distal to exit from the spinal cord) and placing it on platinum recording electrodes. The recording site was approximately 3.5 cm from the proximal stimulating electrodes. The IMCAP produced by stimulation via the proximal stimulating electrodes was first recorded to provide a measure of the total population of myelinated axons just proximal to the repair region. The IMCAP to stimulation via the distal electrodes was then recorded as a measure of the population of myelinated axons that had reconnected across the repair site. The distal IMCAP divided by the proximal IMCAP gives a measure of the ratio of the total myelinated proximal axons having distal connections (49). The ratio is normalized to the total proximal myelinated axon population and expressed as a percentage in Table 1.

A Siegen Neuroscope (Mountain View, CA) was used to trigger a Grass S88 Stimulator (Quincy, MA) with two Model 478A stimulus isolation units. Stimulus parameters were as follows: a constant current with a pulse width of 0.05 msec, amplitude 20 percent above maximal nerve response, and a repetition rate of 2/sec. The nerve signal was recorded and analyzed with the Siegen Neuroscope. The neuroscope allows the storage and retrieval of data; permanent records are made through an attached Hewlett-Packard inkjet printer (Palo Alto, CA). The signal was averaged by recording 50 runs to reduce background noise. The bandwidth was set from 10 Hz to 3 kHz. The maximum pulse amplitude was set at 20 percent above the value needed to stimulate the total population of myelinated axons as measured by the IMCAP algorithm.

De Medinaceli, and more recently, Bain have examined various indices of functional recovery in Walking Track Analysis (52,53). From our examination of these studies, we have begun to employ a Toe Spread Analysis with measurements of first to fifth toe spread (Figure 5). We have found that this parameter shows significant change

Table 1.

Artificial nerve graft (GTMC with collagen type Ic) vs. sutured autograft.

Animal Number	Type of Repair	Months Post-Op	Histology		Axon Count	MFD (μ m)	Physiology Quantitative IMCAP (%)	Functional Analysis Toe Spread mm
			Qualitative Reaction at Graft	Qualitative Organization at Graft				
987R	ANG	9	MIN	GOOD	2503	4.8	72.6	21.0
987L	SAG	9	NONE	FAIR	2514	4.6	79.1	19.5
988R	SAG	9	NONE	FAIR	2438	4.3	83.5	N/A
988L	ANG	9	NONE	FAIR	2176	4.7	90.2	N/A
989R	SAG	9	NONE	FAIR	2878	4.0	78.5	21.5
989L	ANG	9	NONE	GOOD	2050	4.5	75.5	19.5
990R	ANG	9	N/A	FAIR	2921	5.2	87.7	21.0
990L	SAG	9	NONE	FAIR	3606	4.2	80.2	19.5
991R	SAG	9	NONE	FAIR	2476	4.3	94.3	20.5
991L	ANG	9	NONE	FAIR	2588	4.2	81.7	21.0
992R	ANG	6	MOD	FAIR	1588	3.8	90.1	N/A
992L	SAG	6	NONE	FAIR	2495	3.8	82.6	N/A
993R	SAG	9	NONE	FAIR	3327	4.1	83.2	20.0
993L	ANG	9	NONE	GOOD	1860	4.1	53.9	21.0
994R	ANG	6	NONE	POOR	1393	3.7	95.0	N/A
994L	SAG	6	NONE	FAIR	3092	4.3	85.7	N/A
995R	SAG	9	NONE	FAIR	2448	4.3	70.5	20.5
995L	ANG	9	MOD	POOR	2184	3.8	N/A	20.0
996R	SAG	9	NONE	FAIR	2360	4.0	91.2	20.5
996L	ANG	9	NONE	FAIR	2400	4.5	89.1	21.0
997R	ANG	9	NONE	FAIR	2284	3.7	93.7	N/A
997L	SAG	9	NONE	FAIR	3444	3.9	75.4	N/A

Key: ORGANIZATION [excellent, good, fair, poor, or fail]
 REACTION [none, minimal, moderate (mod), or extreme]
 MFD = Mean Fiber Diameter

IMCAP = Integrated Mean Compound Action Potential
 ANG = Artificial Nerve Graft
 SAG = Sutured AutoGraft

following peroneal nerve injury (unpublished data) and is easily measured.

The technique consists of dipping the hind feet of the animal into X-ray paper developer. The animal is then placed into a rectangular box with X-ray film lining the floor, and the animal is allowed to walk the length of the box. Measurements of first to fifth toe spread and print length are easily obtained from the resultant foot prints.

The nerve graft repairs were matched pairs and were

statistically compared for significance by the Student's *t*-test (54,55).

RESULTS

Eleven animals were evaluated at 6 or 9 months post-operation by qualitative histology and quantitative histology and physiology (Table 1).

Figure 5.

Diagram of the rat walking system. On the top, the rat is walking across X-ray film in a Plexiglass walking box. On the right, X-ray film with four footprints is shown. On the bottom, a print with toe spread parameter (first to fifth toe spread) is delineated.

Qualitative histology

Organization at the graft site. The grafts were evaluated for organization at the repair site by determining the number of axons that lay outside the graft on cross-sections (**Figure 6**). In addition, the orientation of the fibers was also used in the determination of the organization of the fascicle. As matched pairs, the SAG were better than the ANG in two cases, while the ANG were better than the SAG in three cases. In six cases, the organization within the ANG and SAG were indistinguishable. Using the Student's *t*-test, no significant difference was found between the two graft types with respect to organization at the graft site ($p > 0.05$).

Reaction at the graft site. The grafts were evaluated for cellular reaction at the repair site in cross-sections stained with H & E. In the majority of cases, minimal or no cellular reaction was found to both grafts (ANG and SAG). No evidence of the original collagen in the ANG

A. ANG at 190 $\times$.

B. ANG at 750 $\times$.

C. SAG at 190 $\times$.

D. SAG at 750 $\times$.

Figure 6 (A, B, C, D). (Right)

Photomicrographs of cross-sections of nerve repair at graft sites in Rat 996 at 9 months. (Stain: Bodian and Aniline blue.)

A. ANG at 190 $\times$. B. ANG at 750 $\times$. C. SAG at 190 $\times$. D. SAG at 750 $\times$.

was noted at 9 months (i.e., no clumping of large particles). There was a moderate reaction in three of the ANG, but in none of the SAG. The other eight matched pairs demonstrated no difference. Using the Student's *t*-test, no significant difference was found between the two graft types with respect to reaction at the graft site ($p > 0.05$).

Quantitative histology

Axon counts. The normal number of axons in the rat peroneal nerve is approximately 2,000 (56). Numbers in excess of 2,000 distal to a repair site are probably indicative of branching by the regenerating axons (**Figure 7**). In nine matched pairs, the SAG had a larger number of myelinated axons just distal to the repair site; in two matched pairs, the ANG had a larger number of myelinated axons just distal to the repair. Axonal count means with standard deviations were found to be SAG=2,825 $\pm$ 464, and ANG=2,177 $\pm$ 443. Statistical analysis of these matched pairs by the Student's *t*-test demonstrated a significant difference (paired *t* value=3.477, $p < 0.01$).

Mean diameters. The mean diameter of the normal rat peroneal nerve is approximately 600 μ m (56). Cross-sections just distal to the repair site were used to obtain mean fiber diameters. In four cases, the mean diameter of the ANG was larger than the SAG; in five cases, the mean diameter of the SAG was larger than the ANG (**Figure 7**, **Figure 8**). Mean diameter means with standard deviations were found to be SAG=4.16 $\pm$ 0.23, and ANG=4.27 $\pm$ 0.51. Using the Student's *t*-test, no significant difference was found between the two graft types with respect to mean fiber diameter (paired *t* value=-0.766, $p > 0.05$).

Quantitative physiology

Our method of physiological evaluation provides a measure of the number of axons regenerated across the repair site (**Figure 9**). In five matched pairs, the SAG had higher percent IMCAPs, and in another five matched pairs the ANG had higher percent IMCAPs. In the remaining matched pair, the nerves could not be evaluated due to technical reasons. IMCAP means with standard deviations were found to be SAG=82.2 $\pm$ 6.7 and ANG=83.0 $\pm$ 12.6. Using the Student's *t*-test, no significant difference was found between the two graft types with respect to quantitative physiology (paired *t* value=0.098, $p > 0.05$).

Figure 7 (A, B, C, D). (Left)

Photomicrographs of cross-sections of nerve repair distal to the graft sites in Rat 996 at 9 months. (Stain: Osmium Tetra Oxide/Phenylene Diamine).

Figure 8A.

Fiber diameter histogram of SAG in Rat 996. The vertical axis is the number of myelinated axons. The horizontal axis is the outside diameter in micrometers. Axons 1 micrometer and smaller were not included.

Figure 8B.

Fiber diameter histogram of ANG in Rat 996. The vertical axis is the number of myelinated axons. The horizontal axis is the outside diameter in micrometers. Axons 1 micrometer and smaller were not included.

Functional evaluation

During the course of this study, a new method of functional evaluation (toe spread analysis) of the repair of the rat peroneal nerve was integrated into our existing methods. Therefore, a subset of the animals (7 animals) in this study also had this functional evaluation (**Figure 5**). In five animals, the SAG yielded a better toe spread pattern than the ANG; in two animals, the ANG yielded a better toe spread pattern than the SAG. Using the Student's *t*-test,

no significant difference was found between the two graft types with respect to toe spread analysis ($p > 0.05$).

DISCUSSION

Indications for nerve grafting vary from gaps of 1 cm to greater than 5 cm or more before a graft would replace an end-to-end repair under tension (2,3,4). Where a graft

Figure 9A.

Compound action potentials in Rat 996 at the proximal and distal repair sites of the SAG. The vertical scale is in microvolts. The horizontal scale is in milliseconds. The distal CAP for the ANG is delayed and spread out compared to the distal CAP for the SAG.

Figure 9B.

Compound action potentials in Rat 996 at the proximal and distal repair sites of the ANG. The vertical scale is in microvolts. The horizontal scale is in milliseconds. The distal CAP for the ANG is delayed and spread out compared to the distal CAP for the SAG.

is indicated, autografts are the preferred method at the present time. The autograft fulfills three major requirements for an ideal nerve graft: 1) it acts as a *passive* conduit for axonal regeneration; 2) it is a *natural* substitute which is immunologically acceptable; and, 3) it is vascularized by the recipient bed as a free graft.

Based on our thought that collagen might be an ideal material for the extracellular matrix of an artificial nerve graft, we tested a commercially available collagen product, Zyderm, in our first artificial nerve graft experiments (22). Zyderm has been clinically used in a large population of patients as a material for repair of subcutaneous defects (23,57). Because of its low antigenicity and easy availability, we studied this compound as a potential extracellular matrix for our first ANG (22). The collagen acted as an effective matrix for regeneration as shown by histological evaluation; however, the electrophysiological evaluations demonstrated statistically inferior results as compared to the sutured autograft (22). **Table 2** summarizes the electrophysiological findings of three of our studies investigating the use of collagen as a matrix for nerve regeneration. Note the dramatic improvement in outcome when switching from Zyderm to collagen Type I with the telopeptides intact. Note also the consistency in the results using SAG repair.

The current study has demonstrated that collagen is a viable extracellular matrix for regeneration comparable to the gold standard SAG (7). Histologically, it was shown that there is minimal long-term reaction to the collagen matrix; moreover, the distal populations of regenerated

axons, are similar in diameter between the ANG and the SAG. However, the SAGs have a statistically significantly higher axonal count than the ANGs. Physiological and functional results demonstrated no statistically significant difference between the ANG and SAG. In spite of the differences in axonal counts, the overall conclusion of the study is that the two repair types result in similar outcomes.

One must bear in mind, however, that the experimental gaps were only 5 mm long. Further work needs to be done to determine how well collagen will work over longer, more clinically relevant distances. This will be determined in nonhuman primates to allow for a more clinically appropriate model. Furthermore, although collagen alone may not be sufficient to allow regeneration across long gaps, collagen is an ideal matrix for the addition of both cellular and noncellular components necessary for the repair of longer gaps.

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Table 2.

A comparison of electrophysiological results from three separate studies.

Study	IMCAP	ANG Composed of
Reference 38	ANG: 32 ± 12 SAG: 73 ± 7	PGA with Zyderm
Reference 38b	ANG: 82 ± 13 SAG: 77 ± 12	PGA with Collagen
Current study	ANG: 83 ± 13 SAG: 82 ± 7	GTMC with Collagen

All studies consisted of 5 mm repair sites.

IMCAP = Integrated Mean Compound Action Potential

ANG = Artificial Nerve Graft

PGA = Polyglycolic Acid

SAG = Sutured AutoGraft

GTMC = Glycolide Trimethylene Carbonate

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