Biodegradable Cuff as an Adjunct to Peripheral Nerve Repair: A Study in Dogs

This study on repair of peripheral nerves has demonstrated the following: (a) the biodegradable cuff is readily placed with conventional surgical armamentarium; (b) tissue tolerance to the cuff is high; (c) fibrosis around the nerve is less with use of the cuff; (d) exact microscopic nerve alignment is difficult to achieve even with magnification and nerve conductive techniques; (e) no increase in conductivity could be demonstrated utilizing the cuff; (f) the inside cuff diameter to nerve diameter does not appear to be optimally 2 to 1 utilizing these biodegradable cuffs; (Cont'd on reverse)
(g) the development of better methods of evaluating peripheral nerve responses is needed.
BIODEGRADABLE CUFF AN
ADJUNCT TO PERIPHERAL NERVE
REPAIR: A STUDY IN DOGS

by

* ROBERT L. REID, M.D., F.A.C.S.
  COLONEL, US ARMY

** DUANE E. CUTRIGHT, D.D.S., Ph.D.
  COLONEL, US ARMY

*** JAMES S. GARRISON, M.D.
  MAJOR, US ARMY

from

* Hand Surgery Section
  Orthopaedic Service
  Walter Reed Army Medical Center

** U. S. Army Institute of Dental Research
  Walter Reed Army Medical Center
  Washington, DC

*** Physical Medicine Service
  Brooke Army Medical Service
  San Antonio, Texas

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the authors and are not to be construed as official or as reflecting the
views of the Department of the Army or the Department of Defense.
For many years experimental and clinical evidence has favored structural support about a peripheral nerve repair.\textsuperscript{1,2} The Ducker-Hayes\textsuperscript{3} silastic anastomotic cuff has been reported to aid longitudinal alignment of neural components, limit the gross local neuroma formation and reduce ingrowth of adjacent scar tissue at the nerve suture site. Several disadvantages have also been shown to exist following use of this non-absorbable cuff material. These include, development of neuroma proximal to the cuff, damage to nerve and adjacent tissue caused by cuff movement when placed adjacent to moving tendons or joints and finally a second surgical procedure is often necessary to remove the nondegradable cuff.

The technique of structural nerve support by cuffing would be markedly enhanced and more widely accepted if the cuff material was biodegradable, light weight and possessed a smooth slick surface allowing tissue movement with little friction or trauma. Such a material has recently been demonstrated in humans and animals. The material, a copolymer of polylactic and polyglycolic acids, is a high molecular weight catabolic product of lactic acid. Properties of this material include controlled rapid degradation,\textsuperscript{4,5,6,7} nontoxic,\textsuperscript{8,9,10,11,12,13} provides a smooth lubricated surface,\textsuperscript{14} is easily fabricated,\textsuperscript{15,16} and can be altered in the operatory.\textsuperscript{16,17}

This is a report of a study in which cuffs of these copolymers were placed about ulna and peroneal nerves in all four legs of 10 adult mongrel dogs. The results were evaluated by clinical response, electro-
myographic observations, nerve conduction studies, and light microscopic examination.

METHOD AND MATERIALS

The biodegradable cuffs were custom made after determining an average size for peroneal and ulna nerves in similar sized dogs. These cuffs were made with an inside diameter twice that of the diameter of the nerve. The cuff wall thickness was 1 mm and the cuff length was 2 1/4 cm.

These cuffs were placed on peroneal and ulna nerves in 10 mongrel dogs weighing between 30 and 40 pounds. One ulna and one peroneal were used as experimental and the opposite nerve was used as a control (Table 1).

The animals were anesthetized using one (1) cc/lb methohexital sodium (Brevital Sodium).** Under standard sterile operating conditions the nerves were individually approached and blunt dissected free of the surrounding tissue. The intact nerve was elevated clear of surrounding tissue and stimulated directly with a bipolar stimulation electrode with 2 gold tips 10 mm apart. Duration of stimulus was 0.1 msec at sufficient amplitude to wake maximal motor response in the limb muscles. A TECA TE-4 direct recording electromyograph with isolated nerve stimulator module was used.* The display was recorded on direct print paper. The recording electrode was a coaxial needle placed in the appropriate muscle distal to the level of nerve section. Ground electrodes were placed between stimulating and recording electrodes. Motor latency times were determined for each individual nerve at the time of surgery prior to nerve section.

*TECA Corporation, 220 Ferris Avenue, White Plains, New York 10603

**Eli Lilly Company, Indianapolis, Indiana
and repair. These studies were repeated for each nerve at the time of harvest in order to document motor recovery. Also at time of harvest electromyograph examination of appropriate limb muscles was done by using a concentric needle electrode.

The surgery and nerve repair was performed using magnification and interrupted 9-0 nylon epineural sutures.

The study was conducted in the U. S. Army Institute of Surgical Research, Brooke Army Medical Center, San Antonio, Texas. The biodegradable cuffs were fabricated and the histopathology evaluated at the U. S. Army Institute of Dental Research, Washington, D. C. 20012.

The nerve samples were harvested on a schedule varying from 8 to 24 weeks postoperatively.

All animals were observed daily. All were active and had gained weight at the time of nerve harvesting. Animal 5 and 8 had a brief erythematous reaction in the wound that responded to antibiotics without overt suppuration.

RESULTS

Tissue Reaction to the Cuff

The early reaction to the biodegradable copolymer has been discussed in several papers. The reaction has been generally one of hydrolysis and phagocytosis with occasional giant cells and with few typical inflammatory cells such as pmls, plasma cells and lymphocytes.

The reaction in this experiment was similar to that referenced above. The eight week samples showed partial breakdown of the cuff into small particles. These particles were surrounded by many phagocytic cells which dispersed slowly as the material hydrolyzed and disappeared in the later time samples. Figures 1 through 4 show the breakdown sequence from 8-12 weeks. The final degradation (disappearance) varied
over a period of 3 weeks when all samples were considered. However, by 8 weeks it was essentially degraded to where tissue proliferated through it and it no longer served as a barrier to the ingrowth of perineural connective tissue (Fig. 1).

The proliferation of fibrous connective tissue between the nerve and the inside of the cuff was in a parallel fashion as shown in Figures 1 & 2. This connective tissue was most likely epineural in origin and had not proliferated from perineural sources outside the cuff.

The distance of the nerve itself from the area of degradation of the copolymer in most cases appeared to be greater than necessary for optimum nerve repair or indeed to prevent an overgrowth of connective tissue around the nerve inside the cuff (Figs. 1-4).

The nerve alignment in both control nerves and experimental nerves was inconstant. Three anastomatic experimental sites are shown in Figures 5,6,7. The alignment in Figure 6 at 8 weeks appears excellent while in Figure 8 at 10 weeks the alignment of the individual nerve fibers was less parallel. Figure 7 at 9 weeks shows considerable overlapping and loss of typical parallel arrangement. However, evidence of traumatic neuroma formation is not present.

Nerve Conduction

The nerve conduction studies first showed evidence of motor return in both the test and control nerves at approximately the 9th week after section and repair. In subsequent weeks there was a tendency for the motor latency time to decrease and for the compound muscle action potential to increase in amplitude and decrease in temporal dispersion. There was a wide variation in the postoperative response from animal to animal. Because of the small number of animals no statistical significance could be placed on the results. Also, no statistical significance
could be documented between the test and control nerves.

Electromyography of the appropriate muscles revealed positive sharp waves and fibrillation potentials from the eighth through the twenty-fourth postoperative weeks. The positive sharp waves and fibrillations decreased in quantity after the sixteenth week. Again, no definite difference could be documented between the test nerves and the controls although there was a general impression of earlier response in the experimental nerves by the electromyographer.

Histopathology Examination

Each nerve (control and experimental) was affixed to white cardboard, the distal and proximal ends were marked and then the cardboard and nerve were placed in 10% buffered formalin. The nerves were examined grossly by bisecting as near as possible through the center of the longitudinal axis of the nerve and repair site. This was not always possible. Both halves of the nerves were oriented and embedded in paraffin, sectioned at 4 microns and stained with Hemotoxylin and eosin, Massons trichrome and Bodians. The slides were examined and recuts ordered where the repair site was not properly bisected. A minimum of 8 slides were examined for each of the following:

a. nerve alignment, judged on a 1 to 3 basis. #1 representing good alignment.

b. thickness of fibrosis around the nerve measured in microns.

c. width of nerve repair measured in microns.

d. noted if sutures and anastomotic site present.

All slides were read blind. The pathologist having never known which were controls and which experimental. All measurements were taken with a Filar Micrometer attachment, calibrated to each lens utilized.
a. Nerve alignment: Eighteen experimental and 18 control peroneal nerves allowed for an analysis of alignment. When the total number given for each nerve were added and an average determined the controls and experimentals were shown to be identical. The ulna nerve was suitable for analysis in 20 experimental samples and in 18 control samples. Again when an average was determined they were identical (see Table 2).

b. Fibrosis: The amount of fibrosis around the nerve was measured, totaled and averaged. In both nerves the experimental showed less fibrosis than the control. Only 2 experimental nerves were judged to have more fibrosis than the corresponding controls. Similarly 8 controls showed more fibrosis than the corresponding experimental (see Table 2).

c. Width of nerve repair: The nerve measurements were done by microscopically selecting the best cross section of nerve and then measuring, at the site of anastomosis, the fibrous connective tissue thickness outside the nerve itself. Although not a highly reliable procedure, it was done uniformly throughout the slide reading the experimental nerves scored lower than the controls in both nerves indicating less fibrosis (Table 2).

d. In 36 nerves studied the pathologist was able to find evidence of the perineural sutures in 30 cases. Thus indicating the exact site of anastomosis. In 3 additional samples the site of anastomosis was identified. In the other three it was difficult to determine if indeed the anastomotic site was exactly identified.
<table>
<thead>
<tr>
<th>DOG IDENTIFICATION</th>
<th>WEIGHT (Kg.)</th>
<th>TIME (weeks)</th>
<th>NUMBER OF SAMPLES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>ULNA Exp Con PERONEAL Exp Con</td>
</tr>
<tr>
<td>SD17</td>
<td>12.3</td>
<td>8</td>
<td>1 1</td>
</tr>
<tr>
<td>5F3</td>
<td>17.9</td>
<td>8</td>
<td>1 1</td>
</tr>
<tr>
<td>5K13</td>
<td>17.5</td>
<td>9</td>
<td>1 1</td>
</tr>
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<td>17.3</td>
<td>10</td>
<td>1 1</td>
</tr>
<tr>
<td>5K29</td>
<td>22.7</td>
<td>11</td>
<td>1 1</td>
</tr>
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<td>12</td>
<td>1 1</td>
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<td>1 1</td>
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<td>19.5</td>
<td>18</td>
<td>1 1</td>
</tr>
<tr>
<td>5F7</td>
<td>11.4</td>
<td>20</td>
<td>1 1</td>
</tr>
<tr>
<td>5F8</td>
<td>9.2</td>
<td>24</td>
<td>1 0</td>
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### TABLE 2

**HISTOPATHOLOGICAL ANALYSIS**

<table>
<thead>
<tr>
<th>Number of Anastomoses Analyzed</th>
<th>Alignment</th>
<th>Width of Fibrosis</th>
<th>Width of Nerve Repair</th>
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<tbody>
<tr>
<td>Peroneal Experimental</td>
<td>18</td>
<td>2.2</td>
<td>510 microns</td>
</tr>
<tr>
<td>Peroneal Control</td>
<td>18</td>
<td>2.2</td>
<td>714 microns</td>
</tr>
<tr>
<td>Ulna Experimental</td>
<td>20</td>
<td>2</td>
<td>886 microns</td>
</tr>
<tr>
<td>Ulna Controls</td>
<td>18</td>
<td>2</td>
<td>1043 microns</td>
</tr>
</tbody>
</table>

*Figures are Averages*
DISCUSSION

The biodegradable cuffs were easily utilized with standard operating aramentarium. The tissue reaction to the cuffs throughout was minimal. Interestingly all average fibrosis measurements were uniformly lower in the experimental nerves. This indicates that the use of the cuff is advantageous and decreases fibrosis and scarring.

No difference in alignment was found between the experimental and control nerves.

The cuffs were essentially degraded by 8 weeks at which time they no longer acted as a barrier to the ingrowth of fibrous connective tissue. Some samples showed a line of phagocytic cells at 12 weeks as the last evidence of the copolymer.

SIZE OF THE CUFF:

Ducker and Hayes\(^3\) state in the dog the maximum direct axonal spanning of the laceration site without connective tissues or neuroma buildup was achieved utilizing tubes whose internal crosssection was twice that of the nerve. In this study an attempt was made to achieve a similar relationship but many of the tissue sections revealed a buildup of connective tissue running parallel to the nerve and located between the cuff and the epineurium. The authors question if this relationship is entirely appropriate and if it could be partially prevented with smaller inside diameter cuffs. Some of our earlier work confirms that when the cuff is snugly applied to the suture site a neuroma develops just proximal to the cuff before biodegradation of the cuff material has occurred. It is obvious that the cuff-nerve diameter relationship is critical and from a practical, clinical point of view difficult to achieve.
CONDUCTION STUDIES:

The authors were unsuccessful in showing any statistical difference electrically between the cuffed versus standard nerve repairs. The equipment either failed in its degree of sophistication to show any difference or the sample was too small or there is no change in the results with the addition of the cuff to the nerve repair. There was a general subjective feeling, on the part of the electromyographer, that there was a general earlier and improved response in the experimental nerves but this was not borne out by the electromyography. More work needs to be done in developing more sensitive equipment so that this experimental point can be clarified.

SUMMARY:

This study on repair of peripheral nerves has demonstrated the following:

a. the biodegradable cuff is readily placed with conventional surgical armamentarium.

b. tissue tolerance to the cuff is high.

c. Fibrosis around the nerve is less with use of the cuff.

d. Exact microscopic nerve alignment is difficult to achieve even with magnification and nerve conductive techniques.

e. No increase in conductivity could be demonstrated utilizing the cuff.

f. The inside cuff diameter to nerve diameter does not appear to be optimally 2 to 1 utilizing these biodegradable cuffs.

g. The development of better methods of evaluating peripheral nerve responses is needed.

Our thanks to Colonel Basil Pruitt, MC and personnel of the United States Army Institute of Surgical Research, Brooke Army Medical Center, San Antonio, Texas.
Figure 1. Eight week sample of the biodegradation of a copolymer nerve cuff.

The biodegradation can be seen as a line of copolymer particles and phagocytic cells (arrows) separating the nerve from the outer connective tissue. The anastomatic line is at lower left (A). X 40.

Figure 2. Ten week sample of the degradation process.

One particle of copolymer remains (A) phagocytes loaded with the final residue of the material can be seen as a line which the copolymer formerly occupied. Tissue reaction is minimal. X 130.

Figure 3. Eleven week samples.

Only a line of phagocytic cells remain. (Arrow) tissue reaction minimal. X 52.

Figure 4. Twelve week sample.

The last evidence of copolymer found in the total study is shown as a line of phagocytic cells (arrow). The connective tissue between nerve A and the phagocytic cells shows little evidence of scarring. X 50.

Figure 5. Nerve alignment at the site of anastomosis at 8 weeks.

The individual nerves are mostly parallel with only occasional nerves growing in an undirected manner A & B. X 130.

Figure 6. Nerve alignment shown at 10 weeks.

It is evident throughout this site of anastomosis that nerve regeneration is haphazard and overlapping. X 64.

Figure 7. Nerve alignment shown at 9 weeks.

This anastomotic site shows poor directional growth. X 64.
BIBLIOGRAPHY


FIGURE 1
FIGURE 5