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A NEW FIBRIN SEAL IN PRIMARY REPAIR OF PERIPHERAL NERVES

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Fibrin sealing, with or without sutures, has become a commonly used technique in human nerve grafting with clinically good results, but is seldom used in primary repair of peripheral nerves. A prospective study was designed to evaluate the incidence of dehiscence in the traditional (Tisseel Kit) and a new "ready-to-use" fibrin seal preparation (Tisseel Duo), and the outcome following fibrin sealing was compared with microsurgical repair of peripheral nerves in rats. No dehiscence was seen in the Tisseel Duo group compared with 20% in the Tisseel Kit group. The electron micrographic evaluation following regeneration showed no significant difference from the microsuture group. Tisseel Duo should therefore be used instead of the traditional fibrin seal preparation when no sutures are used, and may prove to be an alternative to microsuture of peripheral nerves. *Journal of Hand Surgery (British and European Volume, 1994) 19B: 43-47*

Cutaneous sensation of the hand is not usually restored completely after peripheral nerve transection (Önne, 1962; Gelberman, 1992). Young and Medawar (1940) proposed the use of fibrinogen for nerve repair and the first use in man was described in 1942 (Seddon and Medawar, 1942). The fibrinogen was not stable and further development was needed. A stable product was reported in 1972 and the technique was then applied on a greater scale (Matras et al, 1972; Matras, 1985). Since then, the technique has become regular practice in brachial plexus repair (Narakas, 1988) and has been used in peripheral nerves with or without nerve grafting (Matras, 1985). A major advantage has been the reduction in time taken to a quarter or a third (Narakas, 1988). Although some have cast doubt on its use in man (Haase et al, 1986), encouraging results have supported the fibrin glue technique, even when used as the sole agent (Narakas, 1988; Nishihira and McCaffrey, 1989; Bento and Miniti, 1989). Very few animal studies have dealt with the regeneration in sensory nerves, and the outcome has been contradictory (Crutz et al, 1986; Haase et al, 1986). We have been unable to find reports of electron microscopic (EM) evaluation of unmyelinated and myelinated axons following the use of fibrin glue in tributary branches, neither have diameters of myelinated axons following fibrin glue regeneration been reported.

This paper examines the dehiscence rate in the sciatic nerve following neurotomy treated with fibrinogen using either a new "ready-to-use" set (Tisseel Duo including 500 IE Thrombin, Immuno AG, Austria) or a traditional mixture set (Tisseel-KIT including 4 IE Thrombin, Immuno AG, Austria). We have also examined the branches and their axon population following regeneration after neurotomy treated with the most satisfactory fibrinogen application. These results are compared with those following regeneration after a crush lesion to the sciatic nerve, neurotomy treated with microsuture, and with normal controls.

MATERIAL AND METHODS

Animals and surgery

35 3-month-old female Sprague-Dawley rats were anaesthetized with chloral hydrate (30 mg/100 g body weight, intraperitoneal), and the right sciatic nerve was exposed in the thigh. In five rats the nerve was subjected to a crush lesion about 5 mm below the site where it crosses the internal obturator muscle tendon, using watchmakers forceps (2 × 20 seconds). In 25 rats the nerve was cut at the same level with a pair of microscissors. The proximal and distal stumps were carefully joined; in five rats using perineurial 10/0 nylon sutures, in ten rats the nerve was approximated with two pairs of microforceps and a drop of fibrin (Tisseel Duo) placed over the transection site and in ten others a traditional "fibrinogen mixture set" (Tisseel-KIT) was used. A single perineurial 10/0 nylon suture marked the site of the anastomosis. The wound was closed and the rats were allowed to move freely after surgery. Five rats from each of the two groups were examined 2 weeks later and any dehiscence was noted. The other five rats were allowed to survive for 3 months. Five normal rats were used as controls.

Perfusion and collection of specimens

The animals were anaesthetized with chloral hydrate and ventilated with air through a tracheostomy. The heart was exposed and the animals were perfused with 500 ml of Tyrodes solution, followed by 1,000 ml 5% glutaraldehyde in a 300 mOsm phosphate buffer containing 0.1 M sucrose. After perfusion, specimens were taken from the sural nerve (SN), the lateral gastrocnemius nerve motor-innervating the lateral gastrocnemius muscle (LGN) and the posterior articular nerve supplying the knee with sensory innervation (PAN), on the right side. The nerve specimens were post-fixed overnight in glutaraldehyde, osmicated, dehydrated in acetone and embedded in Vestopal W (Berthold, 1968).

Sectioning and electron microscopic examination

Semithin and thin transverse sections were cut with a LKB Ultratome IV, using glass or diamond knives. The sections were taken 30 mm distal to the site of the lesion, corresponding to the knee level. Semithin toluidine blue-stained transverse sections were used for orientation and general evaluation. Thin sections covering the entire cross section of each nerve were collected on one-hole copper grids coated with formvar, contrasted with uranyl acetate and lead citrate, and examined in a JEOL JEM 1200 EX electron microscope. Montages of electron micrographs ($\times 5,000$) were used for counting unmyelinated and myelinated axons and for size measurements of the latter. The numerical differences between regenerated and normal nerves indicated by the axon counts were statistically tested with the Wilcoxon rank-sum test (also known as the Mann-Whitney U-test), for comparison of small groups of non-parametric data (Ejlertsson, 1984).

For each nerve and each experimental situation, measurements of myelinated fibre diameters (D , myelin sheaths included) were made on pictures from three cases. Fibre diameters were calculated from cross-sectional areas determined with Videoplan 2 equipment (Karnes et al, 1977).

RESULTS

General observations

2 weeks post-operatively, five of the animals in each group were re-explored and the repair sites were exposed and examined using the operating microscope. All animals but one in the Tisseel Kit group had by 2 weeks a sciatic nerve in continuity and no neuroma. It was therefore decided that rats from the Tisseel Duo group should proceed for EM evaluation. The general EM pictures following transection and regeneration treated with suture or fibrin seal (Fig 1) showed no differences.

Counts (Table 1 and Fig 2)

Sural nerve (SN)

The normal SN contains an average of 3,821 axons, of which 841 (22%) are myelinated and 2,980 (78%) unmyelinated. Following regeneration after a sciatic crush lesion the total number of axons was 3,195. The number of myelinated axons was 16% higher than normal ($n=985$), but the occurrence of unmyelinated axons was 26% below normal ($n=2,210$). In the sutured group, the average SN total axon number was 3,234. The number of myelinated axons was 41% above normal ($n=1,324$), whereas the occurrence of unmyelinated axons was 36% below the control level ($n=1,910$). In the fibrin sealed group, the average total axon number was 3,047. The number of myelinated axons was 33%

Table 1

| | SN | | LGN | | PAN | |
|--------|---------------|---------------|---------------|---------------|--------------|---------------|
| | M | UN | M | UN | M | UN |
| Normal | 841 | 2980 | 352 | 604 | 86 | 359 |
| (SD) | (± 117) | (± 351) | (± 46) | (± 122) | (± 6) | (± 55) |
| Crush | 985 | 2210 | 498 | 859 | 136 | 764 |
| (SD) | (± 131) | (± 411) | (± 47) | (± 140) | (± 15) | (± 144) |
| Suture | 1324 | 1910 | 513 | 662 | 140 | 368 |
| (SD) | (± 311) | (± 343) | (± 122) | (± 148) | (± 52) | (± 132) |
| Fibrin | 1120 | 1927 | 464 | 590 | 141 | 287 |
| (SD) | (± 289) | (± 236) | (± 101) | (± 133) | (± 47) | (± 144) |

Numbers of myelinated and unmyelinated axons (values within parentheses = SD) in the sural nerve (SN), the nerve to the lateral gastrocnemius muscle (LGN) and the posterior articular nerve of the knee joint (PAN) in normal control animals (NORMAL), in animals which have regenerated over 3 months following a crush lesion to the sciatic nerve (CRUSH), following neurotomy and suture (SUTURE) or following neurotomy and fibrin seal (FIBRIN).

above normal ($n=1,020$) whereas the occurrence of unmyelinated axons is 36% below the control level ($n=1,927$). Statistical analysis shows that the outcome following regeneration in all three situations differed significantly from normal ($P \leq 0.005$), but the outcome following suture did not differ significantly from that following fibrin seal.

Lateral gastrocnemius nerve (LGN)

The normal average LGN contains a total axon number of 956, of which 36% are myelinated ($n=352$) and 64% unmyelinated ($n=604$).

In crushed cases the total number of LGN axons was 1,357. This was an increase of 41% myelinated and 42% unmyelinated axons. Following sciatic neurotomy and suture the average LGN presented altogether 1,175 axons. This was an increase of 45% myelinated and 10% unmyelinated axons. Following sciatic neurotomy and fibrin seal the occurrence of small myelinated axons in the LGN was 31% above normal ($n=464$), and there was an unchanged number ($n=590$) of unmyelinated axons. Statistical analysis of the myelinated axons shows that the outcome following regeneration in all three situations differed significantly from normal ($P \leq 0.005$), and for the unmyelinated axons only the crushed situation differed significantly from normal ($P \leq 0.005$). Therefore the outcome following suture compared with fibrin seal did not differ significantly in either count.

Posterior articular nerve (PAN)

In control animals the PAN contains 446 axons. Of these 19% ($n=87$) are myelinated and 81% ($n=359$) unmyelinated. In crushed cases the total axon number was 900. Both myelinated ($n=136$) and unmyelinated ($n=764$) axons increased, 56% and 113% respectively. Following neurotomy and suture the average nerve had 516 axons, of which 140 (28%) were myelinated and 364

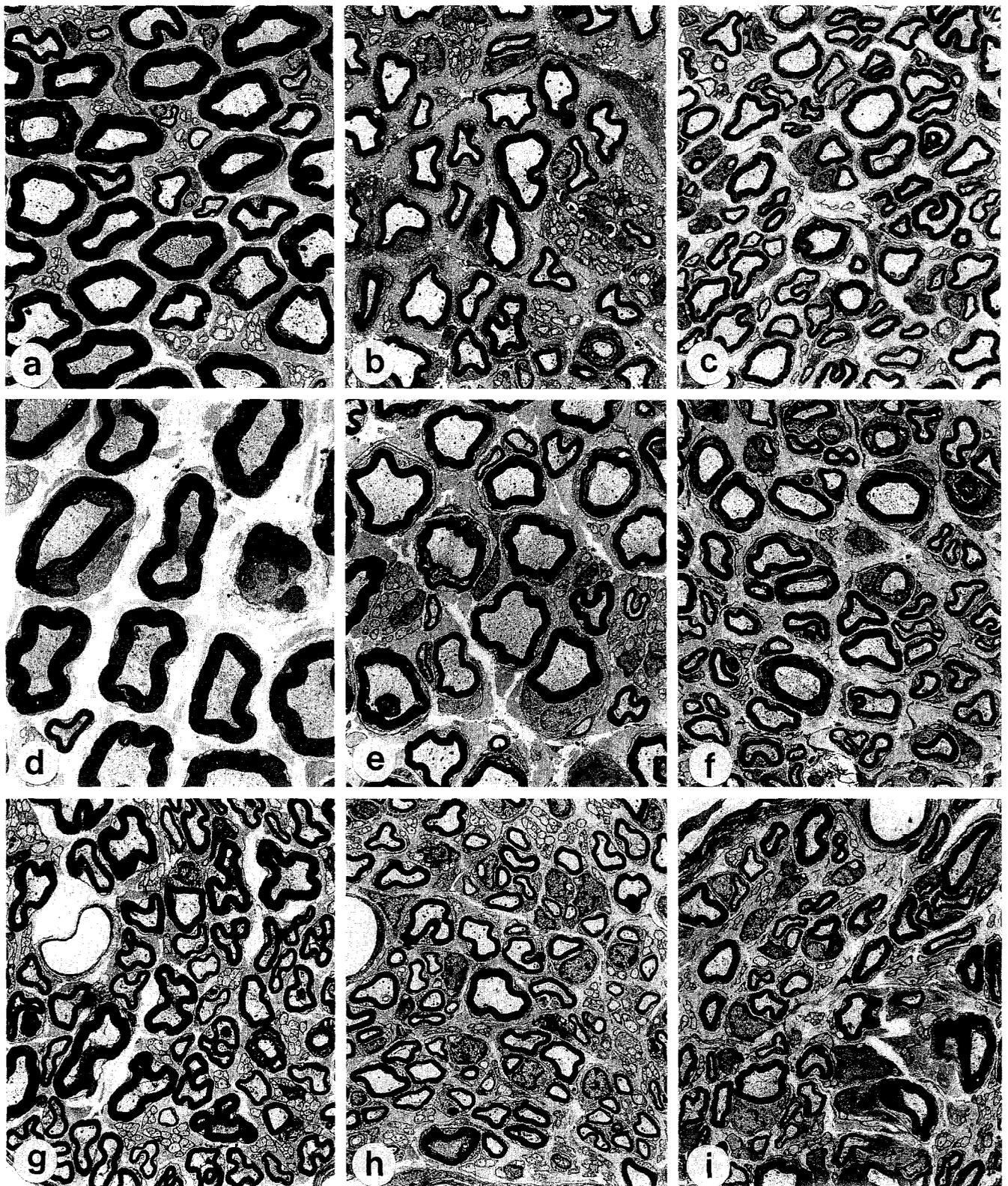


Fig 1 Micrographs ($\times 5,000$) showing axons in the SN (top), LGN (middle) and PAN (bottom) in normals (left row) and 3 months following division and suture of the sciatic nerve (middle row) or approximated by fibrin seal (right row).

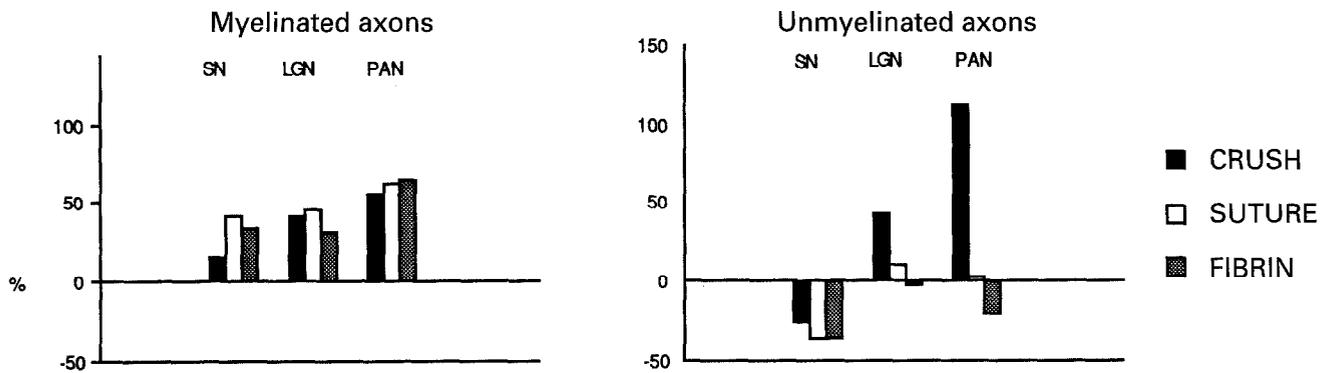


Fig 2 Graphs showing change in numbers of myelinated and unmyelinated axons from the SN, LGN and PAN following a crush lesion (black bars), 3 months following division and suture of the sciatic nerve (white bars) or approximation by fibrin seal (striped bars). No significant differences were seen between animals following division and suture or approximation by fibrin seal, using the Wilcoxon rank-sum test.

(72%) unmyelinated. The number of myelinated axons was 62% higher than normal, but the average occurrence of unmyelinated axons was normal. In the fibrin sealed group, the number of myelinated axons was 64% above normal ($n=141$), but the average number of unmyelinated axons was 21% below normal ($n=287$). Statistical analysis shows that the outcome following regeneration in all three situations differed significantly from normal ($P \leq 0.005$), however the number of unmyelinated axons following suture compared with fibrin sealing did not differ significantly.

Measurements (not shown)

SN

Myelinated fibres in the normal SN reach maximum diameters of about $10 \mu\text{m}$, and there is a broad peak at $2.5\text{--}5.5 \mu\text{m}$. Less than 24% of all myelinated axons have diameters $<3 \mu\text{m}$. Following a crush lesion 28% of all axons had diameters $<3 \mu\text{m}$. In the neurotomy suture and fibrin seal cases, 73% of them had diameters $<3 \mu\text{m}$.

LGN

In controls the LGN exhibits a bimodal myelinated fibre size distribution. The size range reaches $13\text{--}14 \mu\text{m}$, and there is a minimum at $6 \mu\text{m}$. Of the myelinated axons 6.5% have diameters $<3 \mu\text{m}$. In crushed cases 35% of all myelinated axons had diameters $<3 \mu\text{m}$. In divided sutured and fibrin seal cases 70% of all myelinated axons had diameters $<3 \mu\text{m}$.

PAN

Myelinated fibres in the normal PAN present a size range of $1\text{--}8 \mu\text{m}$, and a distinct peak at $3 \mu\text{m}$. Some 40% of all myelinated fibres are $<3 \mu\text{m}$ in diameter. Nerves from crushed cases showed that 72% of all

myelinated axons were $<3 \mu\text{m}$. A similar picture emerged following neurotomy and suture or fibrin seal.

DISCUSSION

The first part of this study examines the dehiscence following neurotomy treated with either a new "ready-to-use" set (Tisseel Duo including 500 IE Thrombin, Immuno AG, Austria) or a traditional mixture set (Tisseel-KIT including 4 IE Thrombin, Immuno AG, Austria). The outcome after apposing the nerve ends with a drop of either of the two and allowing the animals unrestricted movement showed a failure rate in the Tisseel-KIT group of two out of ten (one of five at 2 weeks post-operatively, and one of the five animals that did not proceed to EM investigation at 12 weeks) and none of the ten animals in the Tisseel-DUO group (five at 2 weeks and five at 12 weeks were examined). These findings may explain why the previous results by Haase et al (1986) exhibited such a high failure rate where all distal anastomoses in nerve grafting failed, though the specifications of the fibrin was not mentioned; the "Tisseel-KIT" was probably the only one available. In fact none of the proximal anastomoses failed in the study by Haase. The closer the anastomoses were to the knee joint, the higher the failure rate. The Tisseel-KIT, which may give less tensile strength, would be expected to have a higher failure rate (Young and Medawar, 1940; Matras et al, 1972). Our results show that Tisseel-DUO does not exhibit a failure rate of significance even when the transected nerves are not immobilized, though this precaution is common practice for most peripheral nerve surgery in man. It is therefore interesting to investigate the outcome in branches of the sciatic nerve following treatment with Tisseel-DUO. Many authors have raised the question concerning the fibrinogenic effect of Tisseel in nerve repair and this subject has been investigated by several workers (Crutz et al, 1986; Smahel et al, 1987; Nishihira and McCaffrey,

1987; Herter, 1988; Bento and Miniti, 1989). The negative impact of increased inter-axonal fibrin content is still unclear however.

The present paper examines axonal regeneration in three sciatic nerve branches—the SN, PAN and the LGN. It is apparent from previous work (Hildebrand and Povlsen, 1991) that the SN has a regenerative pattern that differs from that found in the PAN and the LGN, which seem more alike. Following neurotomy and suture, our results show that the SN presents a 41% increase in the number of myelinated axons combined with a 26% decrease in the number of unmyelinated axons. These results concur with previous reports (Jenq and Coggeshall, 1984; Hildebrand and Povlsen, 1991). In the LGN and PAN the myelinated axons are increased following neurotomy and suture (45% and 62% respectively). The unmyelinated PAN/LGN axons, however, which exhibit a significant increase following nerve crush, show a smaller increase after neurotomy and suture (10% and 3% respectively). Following neurotomy and fibrin glue, our results show that the SN deviates from normal in a way resembling that seen after neurotomy and suture, and even the PAN/LGN presents a regenerative pattern that is not significantly different from that seen following suture when the numbers of both unmyelinated and myelinated axons are evaluated. Numerical evaluation of unmyelinated axons following fibrin glue has not been reported before and the few studies which have used light microscopy or low magnification EM evaluation have only examined the myelinated axons of traumatized nerves (Herter, 1988; Cruz et al, 1986; Smahel et al, 1987).

With regard to the effects of sciatic nerve injury on fibre size, our measurements show that in all three nerves, the range is reduced and the spectrum is shifted towards smaller sizes, but no significant differences are found between suture and fibrin seal. We would have expected to find higher numbers of axons with larger diameters in the fibrin seal treated group of the LGN according to previous work showing higher conduction velocities following fibrin seal treatment in muscular branches (Bento and Miniti, 1989). It is possible that the regeneration pattern in our study differs from the facial nerve of the cat which was previously described and which may carry more motor branches among the biggest diameters.

In conclusion, our present observations show that primary peripheral nerve repair with fibrin seal (Tisseel Duo, Immuno AG, Austria) does not give an increased failure rate compared with neurotomy and suture, which has been the case using traditional fibrin glue preparations. The numerical regenerative pattern in branches to hairy skin, joints and muscles does not appear to change significantly when the neurotomy is approxi-

mated by fibrin glue (Tisseel Duo) compared with microsuture. Therefore the new fibrin seal (Tisseel Duo) should be used instead of the traditional fibrin glue preparations when no stay sutures are used, and may even substitute for microsutures in peripheral nerve repair.

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