BRITISH JOURNAL OF PLASTIC SURGERY

Poly-3-hydroxybutyrate (PHB): a resorbable conduit for long-gap repair in peripheral nerves

R. C. Young, M. Wiberg* and G. Terenghi

*Blond McIndoe Centre, Royal Free and University College Medical School, London, UK; and
Department of Hand and Plastic Surgery, University Hospital, Umeå, Sweden

SUMMARY. The repair of peripheral nerve injuries with an autologous nerve graft is still the treatment of choice, although it is widely recognised that the method is associated with donor-site morbidity and a suboptimal functional outcome. An alternative approach is to use a nerve conduit to provide a guidance channel for the regenerating nerve. This study investigates the potential of poly-3-hydroxybutyrate (PHB) nerve conduits to bridge long nerve gaps (up to 4 cm) in a rabbit common-peroneal-nerve injury model. Regeneration was assessed up to 63 days postoperatively, and compared with that achieved using nerve autografts. By 42 days, regenerating axons had bridged nerve gaps of all lengths in groups with nerve autografts and in those with PHB conduits. The regeneration distance increased with time but did not vary with gap length ($P \leqslant 0.009$, 14 versus 21 days, PHB tube 2 cm, 3 cm and 4 cm, Mann–Whitney U-test). At 42 days, the area of immunostained regenerating fibres in the PHB group was greater than that in the nerve autograft group ($P < 0.001$, PHB versus autograft, 21 and 42 days, three-way analysis of variance (ANOVA)). These results suggest that PHB conduits support peripheral nerve regeneration up to 63 days, and that they are suitable for long-gap nerve injury repair. © 2002 The British Association of Plastic Surgeons

Keywords: peripheral nerve regeneration, peripheral nerve repair, nerve autograft, resorbable nerve conduit.

In peripheral nerve surgery the standard method of bridging nerve gaps is by using autologous nerve grafts;[1,2] this provides regenerating axons with a natural guidance channel populated with functioning Schwann cells surrounded by their basal laminae. However, this technique often results in a poor functional outcome and donor-site morbidity, including sensory loss and neuroma formation.[3] In addition, there is a limited amount of donor nerve available. Over the last century, many materials, either natural or man-made, bioresorbable or non-bioresorbable, have been investigated as alternatives to autologous nerve grafts.[2–6]

Our research group has already investigated and reported a number of possible nerve conduits. These include freeze-thawed muscle autograft, freeze-thawed muscle–nerve sandwich autografts, fibronectin mats, polydioxanone (PDS) and poly-3-hydroxybutyrate (PHB).[7–10] The aim of this research has been to develop a bioartificial nerve graft with the benefits of a nerve autograft but without the concomitant morbidity. A suitable material should be biocompatible, probably bioresorbable, beneficial to nerve regeneration and maturation, resistant to scar invasion, and clinically applicable. To date, the most promising material has been PHB. PHB is a natural biological polymer, manufactured as bioresorbable sheets, which can be formed into tubes. The sheets are made up of individual PHB fibres measuring 2–20 μm in diameter, running in a unidirectional orientation. Previous studies in our laboratory have demonstrated that PHB is a possible alternative to an epineural

suture, in terms of neuronal survival, nerve regeneration and inflammatory response, when used as a wrap-around repair technique in sharply divided nerves.[9] More recently, PHB has also been used to repair a 10 mm gap in a rat sciatic-nerve model, where good regeneration and biocompatibility was again demonstrated.[10] A clinical trial is due to start soon using PHB as a wrap-around repair for sharply divided peripheral nerves. However, if PHB conduits are to be accepted into general clinical practice, then they must support regeneration over gaps of more than 2 cm. In order to study nerve regeneration across long gaps,[11] a model in the New Zealand White rabbit was developed using the common peroneal component of the sciatic nerve in the thigh.

Previous work in the rabbit model has demonstrated successful regeneration across 3 cm and 4 cm nerve gaps through natural and artificial conduits.[12–14] However, attempts to bridge a 5 cm gap with freeze-thawed muscle, a collagen conduit and a synthetic conduit were unsuccessful.[4,15] Regeneration across a gap of this size has not been successful without the addition of Schwann cells.[8] The potential advantages of PHB over these other conduits are its physical properties, the fact that it is not of animal or human origin, and that it has already been implanted clinically. The aim of this study was to assess whether PHB conduits would sustain peripheral nerve regeneration across gaps of 2 cm, 3 cm and 4 cm over a short time course. Quantitative morphology was used to determine whether PHB is a suitable material for long-gap nerve repair before proceeding with long-term studies.

Methods

A total of 90 New Zealand White rabbits were studied. Regeneration was assessed at 14, 21, 42 and 63 days in the PHB groups, and at 21 and 42 days in the autograft groups. Fifteen rabbits were studied from each group at each time point, with nerve gaps in the common peroneal nerve of 2 cm, 3 cm and 4 cm ($n = 5$ for each nerve gap). The common peroneal nerve is a mixed motor and sensory peripheral nerve, which, together with the tibial and sural nerves, forms the sciatic nerve in the thigh. The common peroneal nerve, rather than the entire sciatic nerve, was chosen to reduce the chances of autotomy, which is a recognised problem in the rabbit peripheral nerve axotomy model.[16] The conduits were made from sterile sheets of PHB with a unidirectional fibre orientation along their long axes. The overall length of each PHB conduit permitted the proximal and distal nerve stumps to be inset by 2 mm.

Under general anaesthesia and prophylactic antibiotics, the sciatic nerve was exposed in the thigh, and a standard length of the common peroneal nerve was resected to produce the required nerve gap. Under the operating microscope, the nerves were repaired by interposition of a PHB tube (Fig. 1) or an autologous nerve graft. For the nerve grafts we used the resected length of the common peroneal nerve, which was reversed before being secured with microsurgical sutures. All procedures were carried out according to Home Office regulations.

At the time of harvest the nerve repair site was re-exposed, dissected free of the surrounding tissue and excised. In addition to the operated nerves, contralateral unoperated specimens were collected to act as controls. The specimens were pinned out to length and fixed, before being blocked in OCT compound (Tissue-Tek) and stored at $-70\,°C$. We then prepared 15 μm longitudinal frozen sections, which were stained according to the avidin–biotin complex method using nickel enhancement. Regeneration was assessed by staining with monoclonal antibodies to pan neurofilaments (Affiniti, UK; dilution: 1:5000), which stain all types of regenerating fibres. In addition, staining for S 100 (Affiniti, UK; dilution: 1:2000) was used to assess Schwann cell regeneration,
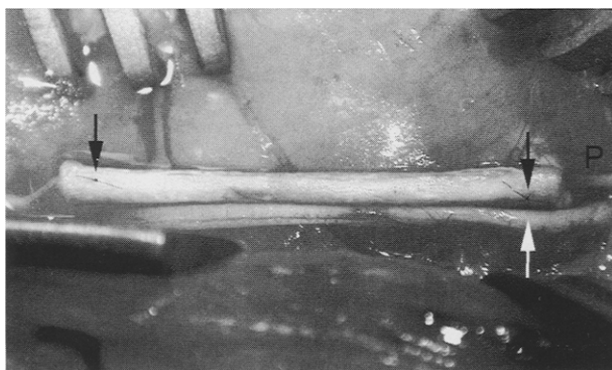

Figure 1—Implantation of a PHB nerve conduit bridging a 4 cm gap in the common peroneal nerve of the rabbit. The conduit is secured with 9/0 epineural sutures (black arrows) and placed in its anatomical position adjacent to the tibial nerve (white arrow). P marks the proximal stump.

and CD 31 (Dako, 1:150) was used to identify endothelial cells and to assess the revascularisation of the conduit.

Pan-neurofilament immunostaining was used to quantify the rate and area of nerve fibre regeneration. The regeneration distance was assessed using light microscopy and a calibrated graticule to measure the most distally stained fibres. The area of immunostaining and the cross-sectional area of the nerve repair site or unoperated control nerve were measured using a digital camera (G Spot) and an image-analysis program (Image-Pro Plus 4, Media Cybernetics, USA). This gave an absolute area of staining, expressed in μm^2, and a percentage area of staining (absolute area/cross-sectional area). The percentage staining allowed a comparison to be made between the wider PHB tube and the narrower nerve autograft in terms of the area of regeneration per unit of area available for regeneration.

Statistical analysis of the results was carried out to determine the effects of gap length and type of repair. To investigate whether there was an effect of gap length, a Kruskal–Wallis one-way analysis of variance (ANOVA) was used to compare the gap lengths for each combination of graft type and day. To investigate the difference in regeneration distance in the PHB tubes between 14 and 21 days a Mann–Whitney U-test was performed for each gap length. To investigate the difference in the regeneration distances between the PHB tubes and the nerve autografts at 21 days a Mann–Whitney U-test was performed for each combination of gap length and day.

The first statistical analysis performed to evaluate the staining area was a three-way ANOVA with the factors day (21 or 42), graft type (PHB or autograft), gap length (2 cm, 3 cm or 4 cm) and their interactions. The interaction term assessed whether the differences between the graft types varied with gap length and day. For the PHB tubes a two-way ANOVA was performed with the factors day (21, 42 or 63), gap length (2 cm, 3 cm or 4 cm) and their interaction. The interaction term assessed whether the differences between the three gap lengths varied with day. Similarly, for the autograft group a two-way ANOVA was performed with the factors day (21 or 42), gap length (2 cm, 3 cm or 4 cm) and their interaction. Following analysis of the initial results, a Student's t-test was used to compare the area of staining in normal nerves with the area of staining in the autograft group at 21 and 42 days.

Results

At all harvest points the PHB tubes were found to be covered in a very thin pseudocapsule, which could be easily removed to allow the PHB tube to be explanted. There was less fibrous tissue at the proximal and distal nerve repair sites of the PHB tubes than at the equivalent locations in the autograft groups. From 21 days onwards it was macroscopically evident that the PHB tube had become well vascularised, as numerous blood vessels encircled the outer surface of the tube. The PHB tubes had not become adherent to the underlying muscles, and there were no anastomotic failures. On microscopic examination, the regenerating nerve fibres were seen to align themselves parallel to the long axis of the PHB

conduits. The fibres grew along the internal aspect of the conduit as well as through the centre of the tube. No regenerating fibres were seen to grow tangentially through the wall of the PHB conduit, although they were freely permeable.

Axonal regeneration distance

The distance reached into the graft by the furthermost pan-neurofilament positive fibres was measured at 14, 21, 42 and 63 days in the PHB group, and at 21 and 42 days in the autograft group. A Kruskal–Wallis one-way ANOVA was performed to compare the gap lengths for each combination of graft type and day. In the PHB tubes at 14 days there were no significant differences in the regeneration distances between any of the gap lengths. At 21 days there were significant differences between the gap lengths in the PHB group ($P = 0.02$, 2 cm versus 3 cm versus 4 cm, Kruskal–Wallis one-way ANOVA). The longest regeneration distance at 21 days was in the 3 cm PHB group, rather than the 2 cm PHB group, making the relevance of this finding uncertain. The regeneration distances measured at 21 days were significantly greater than those measured at 14 days ($P \leqslant 0.009$, 14 versus 21 days, PHB tube, 2 cm, 3 cm and 4 cm, Mann–Whitney U-test) (Fig. 2). By 42 days pan-neurofilament positive fibres were identified in all distal nerve stumps of the PHB groups, indicating that the gaps had been bridged. The regeneration distances were also significantly greater than at the earlier time point ($P < 0.005$, 21 versus 42 days, PHB tube, 2 cm, 3 cm and 4 cm Mann–Whitney U-test). In the autograft groups, pan-neurofilament positive nerve fibres were identified in the distal stump at 21 days in all gap lengths, indicating that the most advanced regenerating nerve fibres had regenerated at least the length of the nerve autograft. The axonal regeneration distance in the autograft group was significantly greater than in the PHB group at 21 days for all gap lengths ($P \leqslant 0.005$, PHB versus autograft, Mann–Whitney U-test).

Axonal regeneration area

The absolute area and percentage area of staining were quantified at a point 5 mm from the proximal nerve repair site at time points of 21 days and longer in both the PHB and the autograft groups. In addition, normal common peroneal nerve was also quantified for the absolute and percentage areas of staining. A three-way ANOVA was performed with the factors day (21 or 42), graft type (PHB or AG), gap length (2 cm, 3 cm or 4 cm) and their interactions. The absolute and percentage areas of staining differed significantly between the graft types ($P < 0.001$, PHB versus autograft, 21 and 42 days) and between the days ($P < 0.001$, day 21 versus 42). For the absolute staining area there was a significant interaction between graft type and day ($P < 0.001$, three-way ANOVA). There were no significant effects of gap length, and no graft–gap, gap–day or graft–gap–day interactions. These results demonstrated that there was a significant difference between the two types of graft, and between time points, but that there was no significant difference between the different gap lengths in each group. As no significant effect of gap length was identified, some of the following data are presented using the total group values rather than the values for each individual gap length.

The absolute areas of staining at 21, 42 and 63 days in the PHB conduits are shown in Figure 3. There was no significant effect of gap length on the absolute area of staining. There were significant differences in the absolute area of staining between the different time points ($P \leqslant 0.001$, 21 versus 42 versus 63 days, two-way ANOVA). There was no significant effect of gap length on the absolute area of staining in the nerve autograft group. A two-way ANOVA was performed to compare the absolute areas of staining in the PHB and autograft groups at 21 and 42 days (Fig. 4). At 21 days there was a significantly greater absolute area of staining in the autograft group than in the PHB group ($P < 0.001$). By day 42 there was a significantly greater absolute area of staining in the PHB group than in the autograft group ($P = 0.004$). There were no differences in the absolute area of staining between the autograft group and the normal control nerves on days 21 and 42 ($P > 0.05$,
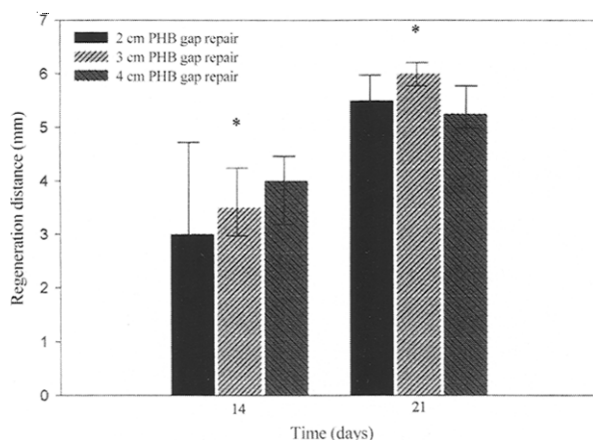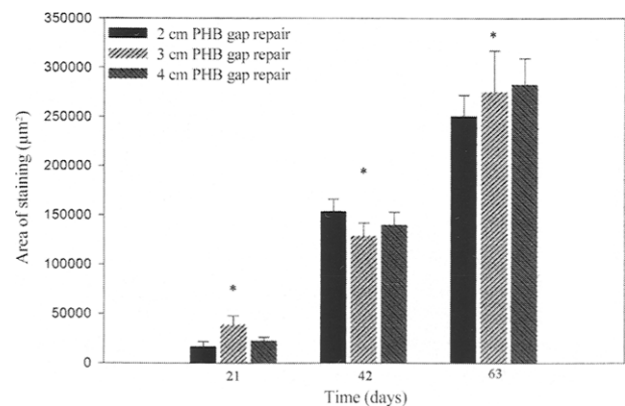

Figure 2—Regeneration distances in PHB conduits at 14 and 21 days. The regeneration distances for 42 and 63 days are not shown because the regeneration front had passed into the distal stump. The data are expressed as mean ± SEM. *$P \leqslant 0.009$, 14 versus 21 days, all gap lengths (Mann–Whitney U-test); $n = 5$ for each group.

Figure 3—Absolute area of staining in PHB conduits at 21, 42 and 63 days. The data are expressed as mean ± SEM. *$P \leqslant 0.001$, 21 versus 42 versus 63 days, all gap lengths (two-way ANOVA); $n = 5$ for each group.
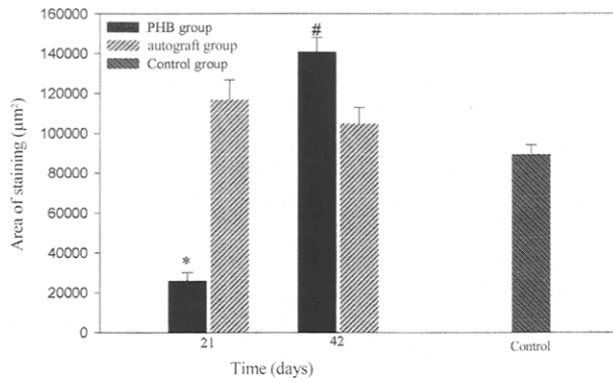
Figure 4—Absolute area of staining in PHB conduits and nerve auto-grafts at 21 and 42 days. The normal unoperated control nerve is shown for comparison. The data are expressed as mean $\pm$ SEM. $*P<0.001$, autograft versus PHB, 21 days; $\#P=0.004$, autograft versus PHB, 42 days (two-way ANOVA).
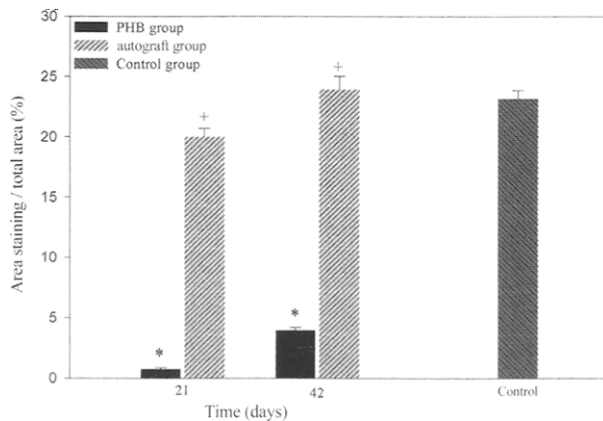

Figure 5—Percentage area of staining in PHB conduits and nerve auto-grafts at 21 and 42 days. The normal unoperated control nerve is shown for comparison. The data are expressed as mean $\pm$ SEM. $*P<0.001$, 21 versus 42 day PHB groups, $+P=0.003$, 21 versus 42 day autograft group (two-way ANOVA); $n=15$ for each group.

t-test). Similarly, there was no difference in the absolute area of staining between days 21 and 42 in the autograft group ($P=0.4$, two-way ANOVA).

The percentage area of staining in the PHB groups increased significantly between day 21 and day 42 (Fig. 5). At both these time points the percentage area of staining was significantly smaller in the PHB group than in the autograft group. This is a reflection of the larger size of the PHB tubes compared with the nerve autografts. In the autograft group there was a significantly larger percentage area of staining on day 42 than on day 21 (Fig. 5). As there was no significant difference in the absolute area of staining, this would suggest that the nerve auto-grafts shrank between day 21 and day 42, perhaps as the inflammatory response settled after Wallerian degeneration in the autograft.

Schwann cells

Sections adjacent to those stained with pan neurofila-ment, and used for the quantification described above,
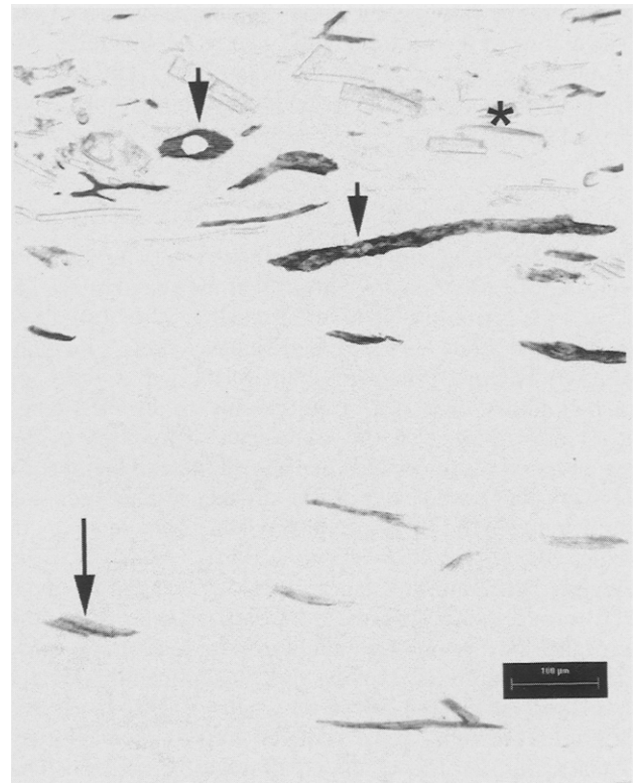

Figure 6—CD 31 immunohistochemical staining for vascular endothe-lium 63 days after implantation. New blood vessels (long black arrow) are aligned longitudinally within the regenerating nerve. PHB fibres (star) are seen along the edge of the regenerating nerve with new blood vessels within the wall of the PHB nerve conduit (short black arrows).

were stained with S 100 to identify Schwann cells. Qualitative assessment showed that the outgrowth of pan-neurofilament positive nerve fibres was mirrored by the outgrowth of S-100 positive cells from the proximal stump. In addition to this outgrowth from the proximal stump, there was a similar outgrowth of S-100 positive cells from the distal stump, resulting in a continuous band of Schwann cells between the proximal and distal stumps by day 42.

Revascularisation

Qualitative assessment for endothelial cells using CD 31 showed that the PHB conduit contained a large number of capillaries by 14 days. These occurred throughout the width of the wall, and appeared to be randomly orien-tated. At a later time point (63 days) longitudinally aligned capillaries could be seen running with the regen-erating nerve (Fig. 6).

Discussion

The last century has seen a periodical interest in the use of nerve conduits for peripheral nerve repair. The pio-neers of nerve conduits used materials such as bone tubes, arteries, muscle and suture material to provide a guidance channel for the regenerating nerve between the proximal and distal stumps.[5,6] The search for alternative

nerve-repair methods declined with the development of microsurgical nerve autografting. This became the treatment of choice for bridging long gaps, and improved the functional outcome for patients undergoing peripheral nerve repair.[1] However, the results are still suboptimal, with very few patients making a full recovery,[17,18] and the technique is associated with substantial donor-site morbidity, including scarring, sensory loss and, sometimes, neuroma formation.[18] It is also becoming apparent that the technical side of peripheral nerve repair has reached an optimal refinement, and additional interventions are needed to improve the outcome.[3,5,17] This has lead to a renewed interest in peripheral nerve conduits and the concept of a bioartificial nerve.

PHB has been used clinically in cardiac surgery,[19] and its physical characteristics suggest that it is also suitable for use as a peripheral nerve conduit. It has been used experimentally as an alternative to direct epineural repair and to bridge short gaps in the rat sciatic-nerve model, with encouraging results.[9,10] In this study, the regeneration distance in PHB conduits was seen to increase over the initial 42 days, after which time regenerating axons were identified in the distal stumps of all animals at all gap lengths. There appears to be an initial lag phase in peripheral nerve regeneration of at least 21 days in this type of conduit, and the start of regeneration is slower than that observed in the rat sciatic-nerve model.[10] This is in keeping with reports that rats have an excellent regenerative capacity, which is more prodigious than in rabbits.[12] At 21 days regeneration had only progressed to a point approximately 5.5 mm distal to the proximal nerve repair site, giving a regeneration rate of 0.26 mm day. By 42 days there were pan-neurofilament positive fibres in the distal stump of the 4 cm gap. Between 21 and 42 days the regeneration rate therefore increased to at least 1.6 mm day. This is slightly slower than the rate observed in the nerve autografts, which crossed the 4 cm gap in less than 21 days, suggesting that the minimum regeneration rate in this model was 1.9 mm day. These values are in keeping with other published data.[13,20] The absolute area of staining in the PHB tubes also increased significantly between day 21 and day 63. At day 21 there was a significantly larger absolute area of staining in the autograft group than in the PHB group, but this situation was reversed by 42 days. At all time points the percentage area of staining, however, was greater in the nerve autografts, reflecting the fact that their cross-sectional area is about six times less than that of the PHB tubes.

The three main principles that lie at the centre of peripheral nerve regeneration are neurotropism, neurotrophism and contact guidance.[6] Neurotropism refers to the ability of the distal nerve stump to release factors that attract regenerating fibres from the proximal stump along a concentration gradient. Neurotrophism refers to the support provided by cellular elements involved in peripheral nerve regeneration and end organs. Regenerating axons randomly sample different pathways; those that make the appropriate choice receive neurotrophic support and go on to mature, whereas those that do not die back. Contact guidance describes the topographical microgeometric cues and physical support provided by the conduit or stroma to the regenerating and maturing axons, as well

as their interaction with cell surface-adhesion molecules, which are present along the appropriate pathways. No differences were demonstrated in the absolute or percentage staining areas at any time between the gap lengths of 2 cm, 3 cm and 4 cm. This suggests that the size of the gap does not influence the neurotropic effect produced by the distal stump on the proximal stump. There is debate about the distance over which the distal stump can influence the proximal stump, but it may be as little as 5 mm.[2,21] The ability of the proximal stump to regenerate may, therefore, be influenced by the other two main factors, contact guidance and neurotrophism. In this model, topographical microgeometric cues are provided by the individual PHB fibres that make up the wall of the conduit. These fibres gradually separate over the first 63 days and occupy some of the original lumen, thus increasing the surface area for cellular alignment and guidance. In addition, the degradation profile of PHB means that it is still present at 63 days, providing physical support to the regenerating nerve. Schwann cells that migrate into the PHB conduit also provide a substrate for the regenerating axons. Neurotrophic factors produced by the end organs are retrogradely transported to the cell body in the intact nerve. After division of a peripheral nerve, retrograde transport ceases as the axons undergo Wallerian degeneration. However, Schwann cells and macrophages, which both play a pivotal role in peripheral nerve regeneration, produce neurotrophic factors, providing limited support and direction to the regenerating fibres.[22-24]

Effective tissue repair requires an adequate blood supply to be established in the area of regeneration. Previous research has shown that neovascularisation occurs immediately ahead of nerve regeneration, and that the regenerating blood vessels also receive contact guidance cues from the surrounding environment.[25] Also, the amount of blood-vessel formation may be increased by the addition of vascular endothelial growth factor, which results in an increase in the amount of nerve regeneration.[26] PHB has been shown to become vascularised soon after implantation, and this will aid regenerative support by delivering the relevant cells and nutrients to the regenerating nerve.

Regeneration in the PHB tubes is slightly slower to start than it is in the nerve autografts. This delay in starting may be due to the lack of Schwann cells in the PHB conduit at the time of implantation, whereas nerve autografts have an inherent population of active Schwann cells. This is also consistent with the continuing regeneration in the PHB groups, shown by the continuing increase in staining area, whereas in the autograft groups the presence of an inherent population of Schwann cells may facilitate regeneration, which is already vigorous by day 21. It is possible that the regeneration rate in the PHB tubes increases once the Schwann cells from the proximal and distal stumps have re-established a line of cellular communication between the end organ and the cell bodies in the dorsal root ganglion.

In conclusion, this study has demonstrated good nerve regeneration, up to 63 days, through PHB nerve conduits over long gaps. Although nerve autograft results in more rapid nerve regeneration, the increasing area of immunostained nerve fibres in PHB conduits over time

suggests that this material supports significant peripheral nerve regeneration. This indicates that PHB may be useful as a resorbable nerve conduit for long-gap repair. However, it is clear that an empty PHB conduit may not be sufficient to sustain optimal peripheral nerve regeneration, and consideration must be given to ways of improving the performance of long PHB conduits, possibly by using exogenous growth factors or cultured cells.

Acknowledgements

References

1. Millesi H, Meissl G, Berger A. The interfascicular nerve grafting of median and ulnar nerves. J Bone Joint Surg 1972; 54A: 727–50.
2. Brunelli GA, Vigasio A, Brunelli GR. Different conduits in peripheral nerve surgery. Microsurgery 1994; 15: 176–8.
3. Mackinnon SE, Dellon AL. Clinical nerve reconstruction with a bioabsorbable polyglycolic acid tube. Plast Reconstr Surg 1990; 85: 419–24.
4. Mackinnon SE, Dellon AL. A study of nerve regeneration across synthetic (Maxon) and biologic (collagen) nerve conduits for nerve gaps up to 5 cm in the primate. J Reconstr Microsurg 1990; 6: 117–21.
5. Fields RD, Le Beau JM, Longo FM, Ellisman MH. Nerve regeneration through artificial tubular implants. Prog Neurobiol 1989; 33: 87–134.
6. Doolabh VB, Hertl MC, Mackinnon SE. The role of conduits in nerve repair: a review. Rev Neurosci 1996; 7: 47–84.
7. Whitworth IH, Brown RA, Doré C, Green CJ, Terenghi G. Orientated mats of fibronectin as a conduit material for use in peripheral nerve repair. J Hand Surg 1995; 20B: 429–36.
8. Whitworth IH, Doré CJ, Green CJ, Terenghi G. Increased axonal regeneration over long nerve gaps using autologous nerve–muscle sandwich grafts. Microsurgery 1995; 16: 772–8.
9. Hazari A, Johansson-Rudén G, Junemo-Bostrom K, et al. A new resorbable wrap-around implant as an alternative nerve repair technique. J Hand Surg 1999; 24B: 291–5.
10. Hazari A, Wiberg M, Johansson-Rudén G, Green C, Terenghi G. A resorbable nerve conduit as an alternative to nerve autograft in nerve gap repair. Br J Plast Surg 1999; 52: 653–7.
11. Terzis JK, Smith KL. Repair and grafting of the peripheral nerves. In McCarthy JG, ed. Plastic Surgery. Philadelphia: WB Saunders, 1990: 630–97.
12. Mackinnon SE, Dellon AL, Hudson AR, Hunter DA. Nerve regeneration through a pseudosynovial sheath in a primate model. Plast Reconstr Surg 1985; 75: 833–41.
13. Fawcett JW, Keynes RJ. Muscle basal lamina: a new graft material for peripheral nerve repair. J Neurosurg 1986; 65: 354–63.
14. Brown RE, Erdmann D, Lyons SF, Suchy H. The use of cultured Schwann cells in nerve repair in a rabbit hind-limb model. J Reconstr Microsurg 1996; 12: 149–52.
15. Hems TEJ, Glasby MA. The limit of graft length in the experimental use of muscle grafts for nerve repair. J Hand Surg 1993; 18B: 165–70.
16. Gutmann E, Guttmann L, Medawar PB, Young JZ. The rate of regeneration of nerve. J Exp Biol 1942; 19: 14–44.
17. Lundborg G. A 25-year perspective of peripheral nerve surgery: evolving neuroscientific concepts and clinical significance. J Hand Surg 2000; 25A: 391–414.
18. Mackinnon SE, Dellon AL. Nerve repair and nerve grafting. In Surgery of the Peripheral Nerve. New York: Thieme Medical Publishers, 1988: 89–129.
19. Duvernoy O, Malm T, Ramström J, Bowald S. A biodegradable patch used as a pericardial substitute after cardiac surgery: 6- and 24-month evaluation with CT. Thorac Cardiovasc Surg 1995; 43: 271–4.
20. Mani GV, Shurey C, Green CJ. Is early vascularization of nerve grafts necessary? J Hand Surg 1992; 17B: 536–43.
21. Politis MJ, Ederle K, Spencer PS. Tropism in nerve regeneration in vivo. Attraction of regenerating axons by diffusible factors derived from cells in distal nerve stumps of transected peripheral nerves. Brain Res 1982; 253: 1–12.
22. Brown MC, Perry VH, Lunn ER, Gordon S, Heumann R. Macrophage dependence of peripheral sensory nerve regeneration: possible involvement of nerve growth factor. Neuron 1991; 6: 359–70.
23. Bunge RP. Expanding roles for the Schwann cell: ensheathment, myelination, trophism and regeneration. Curr Opin Neurobiol 1993; 3: 805–9.
24. Terenghi G. Peripheral nerve regeneration and neurotrophic factors. J Anat 1999; 194: 1–14.
25. Hobson MI, Brown R, Green CJ, Terenghi G. Inter-relationships between angiogenesis and nerve regeneration: a histochemical study. Br J Plast Surg 1997; 50: 125–31.
26. Hobson MI, Green CJ, Terenghi G. VEGF enhances intraneural angiogenesis and improves nerve regeneration after axotomy. J Anat 2000; 197: 591–605.

The Authors

R. C. Young
Giorgio Terenghi PhD, FRCPath

Blond McIndoe Centre, University Department of Surgery, Royal Free and University College Medical School, Royal Free Campus, Rowland Hill Street, London NW3 2PF, UK.

M. Wiberg

Department of Hand and Plastic Surgery, University Hospital Umeå, Umeå, Sweden.

Correspondence to Giorgio Terenghi PhD, FRCPath.