Muscle basal lamina: a new graft material for peripheral nerve repair


The Clayton Foundation for Research, California Division, and the Salk Institute for Biological Studies, San Diego, California, and Department of Anatomy, Cambridge University, Cambridge, England

The suitability of muscle basal lamina as a graft material for the repair of peripheral nerves was investigated. Grafts were prepared by evacuating the myoplasm from muscles excised from rats and rabbits. This produced a material consisting mainly of basal lamina and connective tissue, with the basal lamina arranged as parallel tubes. Rat- and rabbit-derived graft material in 0.5-cm lengths was sutured into rat sciatic nerves, and 4-cm lengths of rabbit-derived graft material were interposed into rabbit sciatic nerves. For controls, 0.5-cm nerve autografts were grafted into rats and 4-cm autografts into rabbits. After 2 to 3 months, the success of the grafts was assessed functionally, electrophysiologically, and anatomically. By all these criteria the basal lamina grafts were as successful as nerve autografts; essentially the same number of axons of the same size grew through both graft types, animals recovered their limb function equally well, and the nerve conduction velocities and relative refractory periods were the same in both groups of animals. In rats, following both basal lamina and nerve autografts, the number of axons distal to the grafts was approximately the same as that proximal to them, but axon diameter and speed of conduction were significantly less than normal. The authors conclude that muscle basal lamina grafts are as effective as nerve autografts for repairing severed rat or rabbit peripheral nerves, and suggest that grafts prepared in this way may prove to be useful for nerve repair in humans.

KEY WORDS: nerve regeneration, nerve repair, basal lamina, peripheral nerve

When repairing an injury to a peripheral nerve it is often necessary to bridge a gap between the proximal and distal ends of the damaged region with a graft. Regenerating axons are then able to grow from the proximal stump of the nerve through the graft and into the distal stump. In current practice, the graft material most often used is a live nerve autograft taken from another peripheral nerve, usually the sural nerve. This procedure has several disadvantages. If the injured nerve is of large diameter, as are the major nerves of the arm and leg, the graft may be too thin to join the nerves adequately. Several parallel lengths of graft, therefore, have to be sutured into the gap, and this can lead to excessive scarring and poor axonal regeneration; if the injuries are severe there may not be enough suitable graft material available. Also, removing the graft involves a large incision, and inevitably results in a loss of sensation in the denervated area. Alternative types of graft, ideally prepared in advance of placement, would therefore be useful.

When axons regenerate within peripheral nerves they are usually confined to the endoneurial tubes, which consist of Schwann cells and their associated basal lamina. Electron microscopic studies have shown that the growth cones of such regenerating axons are often attached to the inner surface of the basal lamina. In tissue culture, axons grow particularly well on substrates of two of the major basal lamina components, laminin and fibronectin, and laminin gel has been found to enhance the regeneration of axons through chambers inserted between two cut ends of a peripheral nerve. Theoretically therefore, a suitable nerve graft material might be made up of many basal lamina tubes lying parallel to one another.

Skeletal muscle presents just such a tissue, for each muscle fiber is surrounded by a thick layer of basal lamina. If a muscle is damaged in situ, the cytoplasm and plasma membrane degenerate, but the basal lamina tubes remain intact. It is also possible to remove the cytoplasm and plasma membrane from the basal membrane of excised muscles either by freezing and thawing or by various chemical and/or mechanical
Muscle basal lamina graft for nerve repair

treatments;\textsuperscript{15,28} this has also been recorded by one of us (Keynes, unpublished observations). It has recently been found that regenerating peripheral nerve axons, when forced to grow into degenerating rodent skeletal muscle, show a strong preference for growth inside the basal lamina tubes,\textsuperscript{9,10} the axons grow extensively along the length of the original muscle fibers, and most remain confined to the tube that they first entered.

In the present study, we have investigated the potential usefulness of muscle basal lamina grafts for peripheral nerve repair, using them to repair transected sciatic nerves in rats and rabbits. We find that axonal regeneration in nerves repaired in this way, as assessed both functionally and morphologically, is as extensive as in nerves repaired with nerve autografts.

Materials and Methods

Animal Preparation

Male and female Sprague-Dawley rats, 6 to 8 weeks old, were anesthetized by intraperitoneal injection of 3.5\% chloral hydrate. The sciatic nerve on one or both sides was exposed in the region of the greater trochanter and transected. A graft 0.5 cm long, derived either from muscle or nerve (see below), was then sutured to the epineurium of the proximal and distal nerve stumps with 8-0 virgin silk. Three rats received live nerve grafts, and 10 received muscle basal lamina grafts (20 grafts).

Female 6-month-old New Zealand White rabbits were anesthetized with intravenous ketamine and Nembutal (sodium pentobarbital). The sciatic nerve was exposed as in the rats. For basal lamina grafting, a 2-cm graft on one side was exposed in the region of the greater trochanter and transected. A graft 0.5 cm long, derived either from muscle or nerve (see below) was then sutured to the proximal and distal nerve stumps, using 8-0 virgin silk for each of the two or three separate fascicles of the nerve. For nerve grafting, the sciatic nerve in one leg was cut in two places 4 cm apart; the nerve was then rejoined with virgin silk sutures to each fascicle. Following closure, the denervated limb was bandaged to prevent the development of pressure sores. Three rabbits received nerve autografts, and eight received basal lamina grafts (to one leg only).

Graft Preparation

Live Nerve Grafts. In rats, a 0.5-cm length of sciatic nerve was resected on one side and immediately grafted on the other side. In rabbits, one sciatic nerve was cut in two places 4 cm apart, and then rejoined, without removing the 4-cm segment from the limb.

Muscle Basal Lamina Grafts. In rats, soleus muscles were emptied of cytoplasm and plasma membrane by the following procedure, adapted from the method of Wallis, et al.\textsuperscript{25} The muscle was immersed in iced distilled water for 15 minutes and rinsed in 10 mM CaCl\textsubscript{2}/2 mM imidazole (pH 7.2); it was then soaked in the same mixture for 2 hours. After rinsing in 30 mM KCl/3 mM Tris (pH 8.2), the muscle was immersed in the same solution overnight before being transferred into 0.09\% triethanolamine (pH 8.7 to 9.0) for 1 to 2 hours at room temperature. Final evacuation was achieved by compressing the muscle between two flat surfaces.

An alternative, simpler method of evacuation of cytoplasm consisted of alternate freezing and thawing in liquid nitrogen and saline three times, followed by osmotic evacuation in distilled water at room temperature, aided by mechanical pressure. Unfortunately, the grafts prepared in this way tended to fragment.

In rabbits, adductor magnus muscles were treated in the same manner as the rat soleus muscles. This resulted in the evacuation of only about 50\% of the cytoplasm; the technique for evacuating this type of muscle needs to be improved.

Electrophysiological Studies

Each rat was anesthetized with intraperitoneal chloral hydrate (1 ml/100 gm body weight of a 3.5\% solution). The graft was exposed and transected 2 cm proximal and 2 cm distal to the grafted region. The nerve segment was then cleaned of adherent fat and blood vessels and placed in a recording chamber. The electrodes were positioned with the stimulating electrode and first recording electrode proximal to the graft and the second recording electrode immediately distal to the graft. Compound action potentials were recorded using Tektronix type 122 preamplifiers, connected to a Tektronix oscilloscope.* Conduction speed through the graft in the fastest fibers was estimated from the delay between the upstrokes of the compound action potentials measured at the two recording electrodes. Relative refractory periods and stimulus strength-duration curves were also recorded.

Histological Study

At varying times after grafting, the animals were killed and perfused with saline. Tissues were then fixed by perfusion of the animal with a solution of 2\% paraformaldehyde and 0.5\% glutaraldehyde in 0.1 M phosphate buffer. Segments 1 to 2 mm in length were removed from the following regions of each operated nerve: 1) 2 to 3 mm proximal to the proximal graft suture line in rats, and 5 mm in rabbits; 2) midway along the graft; and 3) 2 to 3 mm distal to the distal graft suture line in rats, and 5 mm in rabbits.

After post-fixation in a solution of 4\% paraformaldehyde and 2\% glutaraldehyde in saline, the nerve segments were embedded in Spurr’s resin. Transverse semithin sections, 1 \textmu m thick, were cut and stained with toluidine blue; transverse ultrathin sections were stained with lead citrate and uranyl acetate and examined in a Zeiss electron microscope. The number of myelinated axon profiles in a semithin section was estimated by the following procedure.

* Preamplifiers (Type 122) and oscilloscope manufactured by Tektronix, Inc., Beaverton, Oregon.
FIG. 1. Photomicrographs of graft sections. Bars = 50 µm. a: A section from the graft region of a rat basal lamina graft in a rat sciatic nerve, after 2 weeks' regeneration. The zinc/osmium-stained regenerating axons are mostly running inside the basal lamina tubes and are therefore growing parallel to the axis of the nerve and to one another. × 200. b: The distal stump of the sciatic nerve from the same animal as in a. This section is taken from near the graft-nerve boundary. The black smudges are degenerating myelin. A few axons can be seen regenerating into the nerve. × 200. c: This silver-stained section is taken from the graft region of a rabbit sciatic nerve that had been repaired with a rabbit-derived basal lamina graft. The animal was killed 3 weeks after operation. Axons are clearly seen within the basal lamina tubes of the graft. This section was taken from the distal end of a 4-cm graft, so the axons had grown at least 2 mm/day. × 200. d: A zinc/osmium-stained section from the graft region of a rat in which a live nerve autograft had been sutured into the sciatic nerve 2 weeks previously. Stained axons are seen among the degenerating myelin. × 300.

The outline of the section was drawn with a drawing tube and the area was determined with the aid of a computerized digitizing tablet. The axons were counted in a representative 3000-sq µm area of the section, from which the axon density, and thus the total axon count for the whole section, was estimated. To obtain the average diameter of myelinated axons in a section, a representative area was chosen, and the widest diameter (inclusive of myelin sheath) of each of 100 axons within it was measured with an eyepiece graticule. The remaining segments were processed for silver staining. For this they were post-fixed in 10% buffered neutral formalin, paraffin-embedded, sectioned longitudinally at 15 µm, and stained by a Bielschowsky silver staining procedure.

In some rats the operated nerves were stained with zinc iodide/osmium tetroxide. The animals were killed, and the grafted regions dissected and placed in a freshly prepared solution of 6 ml zinc iodide to 1.75 ml 2% osmium tetroxide. The zinc iodide solution was made by combining 5 gm iodine with 15 gm powdered zinc in 200 ml water. The tissue was incubated at 55°C for 90 minutes, washed with distilled water, and embedded in a gelatin-albumin mixture (90 gm Fisher albumin A388 + 60 gm Sigma albumin A5253 in 200 ml 0.1% PO₄ buffer, and 1.5 gm gelatin in 100 ml PO₄ buffer mixed together, then 60 gm sucrose added), set by the addition of 1 part in 10 of 25% glutaraldehyde. Longitudinal sections were then cut at 50 µm on a freezing microtome.

Results

We found that by both anatomical and functional criteria, sciatic nerves repaired by muscle basal lamina grafts regenerated as well as those in which live nerve autografts were used.
Muscle basal lamina graft for nerve repair

**Fig. 2.** Studies of a rat-derived basal lamina graft in a rat sciatic nerve after 3 months' regeneration. 

a: Photomicrograph of a semithin section from the center of the graft. Many myelinated axons are seen, divided into fascicles. × 300. Bar = 50 μm.  

b: Electron micrograph of the area illustrated in a. Large myelinated axons are present, with groups of unmyelinated axons invested in Schwann cells interspersed between them. The lower arrow points to a layer of homogeneous lightly staining matrix, which is probably the muscle basement membrane. The upper arrow indicates layers of cell processes, probably from fibroblasts, which are frequently seen investing the layers of basement membrane. × 4000. Bar = 2 μm.  
c: Photomicrograph of a semithin section from the sciatic nerve distal to the grafted region. Myelinated axons of around the same size as in the graft region are seen. × 300. Bar = 50 μm.  
d: Electron micrograph from the same region as in c. Both myelinated and unmyelinated axons are seen in about the same proportions as in b. × 4000. Bar = 2 μm.

**Muscle Basal Lamina Grafts**

**Rat Studies.** The earliest time after grafting at which axon growth was examined was 14 days, in preparations stained with zinc iodide/osmium tetroxide. In these cases, large numbers of axons had grown into the grafts, running parallel to the long axis of the muscle fibers and growing inside the basal lamina tubes of the graft (Fig. 1a). Many axons had reached the distal stump of the nerve, but they had not extended more than about 100 μm into it (Fig. 1b).

The remaining animals were killed between 2 and 3 months after grafting. By this time both the grafts and the distal stumps contained large numbers of thick myelinated axons (Fig. 2). The regenerated axons were significantly thinner (p < 0.001) than axons in the nerve proximal to the repair (Table 1), as were their myelin sheaths (Figs. 2 and 3). Within the grafts, axons were usually fasciculated, with one or two major fascicles in the center of each graft and several smaller ones at the

**Fig. 3.** Photomicrograph of a semithin section from the sciatic nerve of a rat that had received a basal lamina graft, taken from the nerve proximal to the graft so as to show the non-regenerated portion of the nerve. × 300. Bar = 50 μm.
edges. In addition, in some cases there were axons at the edges of the grafts that grew tortuously, often appearing to follow blood vessels. The routes that the axons took across the graft boundaries were somewhat variable, and seemed to depend on how well the graft had been aligned at the original surgery. In the best cases, the regenerating fibers grew in a straight line directly from the proximal stump to the graft and from the graft to the distal stump, but in those cases in which the surgery had been less successful there was a neuromatous region at the graft boundary. It was noticeable that, once in the graft, the axons grew straight through it, staying parallel to one another. Each graft was plentifully supplied with blood vessels throughout its thickness.

Rabbit muscle basal lamina grafted into rats appeared very similar to grafts derived from rat muscle. The rabbit adductor magnus muscle has larger, more segregated fascicles of muscle fibers than the rat soleus muscle, and this produced grafts containing more individual axon fascicles than rat muscle grafts (Fig. 4), but the main features were otherwise the same. The appearance of the axons in the distal stumps was also the same as for rat muscle grafts.

In each rat, the number of myelinated axons in the distal stump of the repaired nerve was calculated for comparison with the number proximal to the graft. As seen in Table 1, the number of axons distal to the repair was the same as the number proximal to it for the animals which received basal lamina grafts, whether of rat or rabbit origin. Combining this result with the appearance of the silver-stained material, it is likely that the great majority of axons had regenerated successfully through each graft into the distal stump of the nerve.

**Rabbit Studies.** The muscle basal lamina grafts into rabbits gave essentially the same results as grafts of the same material into rats. Two rabbits were studied 3 weeks after insertion of 4-cm basal lamina grafts, and in silver-stained sections, axons were seen that had regenerated the full length of each graft within this time (see Fig. 1c). After 3 months of regeneration, the axons were generally slightly thinner than in the rat experiments in both the grafts and the distal stumps, and many of the axons were poorly myelinated or still unmyelinated (Fig. 5). It was also noticeable that the axons were thicker and stained more intensely with silver in the proximal than in the distal parts of the grafts. In the region of the graft, the axons were broken up into clear fascicles and were separated by areas of connective tissue. The main differences between the appearance of the grafts in the rats and in the rabbits are probably explicable on the basis of graft length: the rabbit grafts were eight times as long as the rat grafts, and, not surprisingly, their distal regions had not reached the same degree of maturity. In rabbits, many of the regenerated axons were still unmyelinated after 2 to 3 months of regeneration (Fig. 5), making counts of myelinated axons difficult to interpret. However, rough axon counts indicated that the number of axons regenerating through successful basal lamina grafts was roughly the same as the number that regenerated through successful live nerve autografts (see below).

**Live Nerve Grafts**

All rats that received nerve autografts were examined 2 to 3 months after operation. In general, the microscopic appearance of the grafts was similar to the basal lamina grafts. In the best cases, the axons had grown

---

TABLE 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before Graft</th>
<th>Rat Basal Lamina</th>
<th>Live Nerve</th>
<th>Rabbit Basal Lamina</th>
</tr>
</thead>
<tbody>
<tr>
<td>no. of axons</td>
<td>11,594 ± 2175</td>
<td>14,217 ± 1813</td>
<td>10,542 ± 686</td>
<td>12,083 ± 1335</td>
</tr>
<tr>
<td>no. of rats</td>
<td>3</td>
<td>14</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>diameter of axons (μm)</td>
<td>6.5 ± 2.7</td>
<td>3.7 ± 1.6</td>
<td>3.9 ± 1.9</td>
<td></td>
</tr>
<tr>
<td>control tissue</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mid graft</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>post-graft</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no. of rats</td>
<td>4</td>
<td>5</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

*Values are means ± standard deviation. The number of axons indicates those distal to the graft.*
Muscle basal lamina graft for nerve repair

straight through the graft and into the distal stump. They were well myelinated, although significantly (p < 0.001) thinner in the graft and distal stump than in the nerve proximal to the repair (Fig. 6). The number of axons distal to the repair was the same as that proximal to it, and was essentially the same as in basal lamina grafts (see Table 1).

In rabbits, the results of nerve grafting also seemed to be very similar to those of basal lamina grafting (Fig. 7). The density and size of myelinated axons in the distal stump of the nerve were similar in the two groups. The axons in the middle of the graft, however, were not grouped into such clear fascicles in the nerve-grafted animals as in the basal lamina-grafted animals.

Physiological Testing

Physiological function was tested in rats that had undergone sciatic nerve repair with basal lamina or live nerve grafts 3 to 4 months previously; results from these tests were compared to those in a series of normal rats. In each case, we measured the speed of conduction through the graft in the fastest fibers and the relative refractory period, and plotted the stimulus strength-duration curve. The latter was not particularly informative, being nearly the same in normal and experimental animals, so only findings from the first two tests are reported (Table 2).

Axons that had regenerated through basal lamina grafts were capable of conducting action potentials as well as those that had regenerated through live nerve grafts. However, in both cases the axons conducted significantly more slowly than normal, reflecting their smaller average diameter. The refractory periods were not significantly elongated in the post-graft as compared to the pre-graft nerves; similar findings have been reported by previous authors.3,17,22

Fig. 5. Studies of a rabbit-derived basal lamina graft in a rabbit sciatic nerve after 3 months' regeneration.

a: Photomicrograph taken from the center of the graft, from a region selected to show the very marked fasciculation of regenerating axons in these cases. The cross-sectional area of the axon-containing graft is high in the middle of these grafts to accommodate the rather widely dispersed axons, but at the distal end of the graft the axons appear to converge on the distal stump. We believe that this loose fascicular pattern reflects the properties of the muscle from which we made our grafts (see text). × 300. Bar = 50 μm. b: Electron micrograph from the same region as a. There are large myelinated axons with unmyelinated fibers interspersed among them. × 4000. Bar = 2 μm. c: Photomicrograph of a semithin section taken about 5 mm distal to the graft. The regenerating axons have converged in the distal stump, so the axon density is much higher than in the mid graft region. × 300. Bar = 50 μm. d: Electron micrograph from the same region as c showing a few large myelinated axons, with unmyelinated axons around them. The proportion of unmyelinated axons is higher than in our rats with basal lamina grafts. × 400. Bar = 2 μm.
FIG. 6. Studies of a rat sciatic nerve autograft after 3 months' regeneration.  a: Photomicrograph of a semithin section from the mid graft region of the nerve. × 300. Bar = 50 μm. b: Electron micrograph from the same region as a. There are large myelinated axons present, with unmyelinated axons among them. × 4000. Bar = 2 μm. c: Photomicrograph of a semithin section taken from the nerve distal to the repair. The axons are of a similar size and density to those in the region of the graft. × 300. Bar = 50 μm. d: Electron micrograph of the nerve distal to the graft. The appearance is similar to that in b. × 4000. Bar = 2 μm.

TABLE 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal Nerve</th>
<th>Basal Lamina Grafts</th>
<th>Live-Nerve Grafts</th>
</tr>
</thead>
<tbody>
<tr>
<td>conduction speed (m/sec)</td>
<td>31.6 ± 4.4</td>
<td>17.4 ± 2.9</td>
<td>17.0 ± 2.6</td>
</tr>
<tr>
<td>no. of rats</td>
<td>10</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>relative refractory periods (msec)</td>
<td>7.9 ± 1.5</td>
<td>7.1 ± 1.9</td>
<td>6.8 ± 1.4</td>
</tr>
<tr>
<td>pre-graft</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>post-graft</td>
<td>8.0 ± 1.6</td>
<td>6.8 ± 1.6</td>
<td></td>
</tr>
<tr>
<td>no. of rats</td>
<td>10</td>
<td>8</td>
<td>4</td>
</tr>
</tbody>
</table>

* Values are means ± standard deviation.

Functional Recovery

Many factors are involved in the return of limb function following nerve grafting. In particular, axons that regenerate must be guided to their correct targets. In this experiment we wished to concentrate simply on the ability of our two types of nerve grafts to support axonal regeneration. However, we did perform brief functional examinations of our experimental animals. These showed that the anatomical and physiological evidence of nerve regeneration detailed above was matched by a substantial degree of recovery of limb function in animals with grafted nerves. Rats with nerves repaired by either basal lamina or nerve grafts regained good control of their knee and ankle joints within the 3- to 4-month regeneration period, and in about half of the rats the ability to spread the toes had also returned. The rats on which we performed live nerve autografts always had one unrepaired sciatic nerve from which a 0.5-cm length had been excised, and the limbs supplied by these sectioned nerves showed no signs of functional recovery. As expected, the rabbits had a less complete return of function in their much longer legs, but in the 3-month period of the experiment they regained sensation over much of the limb, good control of the knee joint, and in most cases partial control of the ankle joint.
Muscle basal lamina graft for nerve repair

Discussion

The success or failure of a peripheral nerve repair is determined by the number of axons that regenerate through the repair site into the distal stump, and by the proportion of those axons that reach their correct targets and make connections there. Whether axons regenerate to their correct targets or not is only slightly influenced by surgical technique; many axons are inevitably misdirected, particularly when the site of the nerve repair is some distance from the target tissues. Solving this problem will involve finding ways to make regenerating axons recognize guidance cues similar to those that directed them to their targets during embryonic development. At present, therefore, the main aim in surgical nerve repair must be to ensure that the maximum number of functional axons grow through the site of the nerve repair into the distal stump. In making a preliminary assessment of the suitability of muscle basal lamina grafts for peripheral nerve repair, therefore, we have concentrated on the main modifiable factor: how many functional axons grow through nerve grafts made of this material, and how do the results compare with those of nerve autografts, the usual material of choice for nerve grafting?

A successful graft material must have the following properties: 1) regenerating axons must grow into it, extend through it, and grow beyond it into the distal stump at least as well as they do through nerve autografts; 2) having grown through the graft, the axons must mature to something approaching their normal size, they must become properly myelinated, and they must conduct action potentials normally; 3) the graft material must be immunologically compatible with the host; 4) the graft must become fully vascularized; and 5) there should be minimal disorganization of axons within the graft.

We find that, in rats and rabbits, evacuated muscle basal lamina grafts meet these criteria at least as well as do nerve autografts. We will discuss these five points in order.

1. Axons grow into and through basal lamina grafts rapidly. We examined 0.5-cm rat grafts 14 days after operation, and found that a large number of axons had grown through the grafts into the distal stump. We sectioned two repaired rabbit nerves 3 weeks after operation, and found that some axons had reached the distal stump within this time, corresponding to a rate of axonal growth of at least 2 mm/day.

2. In both rats and rabbits, the anatomical and functional results of nerve repair were as good in the animals that received a basal lamina graft as in those that had a nerve graft. The number and size of the myelinated axons that had regenerated into the distal stump of the nerve were the same in the two graft types, and in our rat experiments the physiological function of the repaired nerves was as good in the basal lamina grafts as in the nerve grafts. This implies that axons grew through the grafts and subsequently enlarged and matured, and that Schwann cells migrated from the nerve stumps to

FIG. 7. Studies of a live nerve autograft in a rabbit after 3 months’ regeneration. a: Photomicrograph of a semithin section from the mid graft region. × 300. Bar = 50 μm. b: Photomicrograph of a semithin section from the nerve about 5 mm distal to the graft. × 300. Bar = 50 μm. c: Electron micrograph from the same region as b. There are many unmyelinated and a few myelinated axons. × 4000. Bar = 2 μm.
myelinate the regenerated axons. These anatomical and physiological results were accompanied by a return of limb function which was equally good in basal lamina- and nerve-grafted animals.

3. Basal lamina grafts do not seem to undergo immune rejection by their hosts, which is what one might expect from a material that contains no living cells. In our operations, graft material was taken from a donor animal unrelated to the eventual host; no evidence of rejection was seen. We were not able to evoke the rabbit muscle completely, and this caused some local inflammation while the dead myoplasm was removed. At 3 weeks after the operation this was still just evident, but in animals killed 2 months after operation there was no continuing sign of local inflammation. In order to see whether grafts derived from one species would be successful in another, we grafted rabbit muscle basal lamina into rat sciatic nerves. By our anatomical criteria, these repairs were just as satisfactory as those made with basal lamina grafts of rat origin, or indeed as the nerve autografts. It should be possible, therefore, to prepare grafts from animal tissue for use in humans. The precedent for the use of grafts of acellular animal tissue in human surgery has long been established; for example, porcine heart valves are used to replace damaged human valves.

4. Basal lamina grafts are rapidly vascularized. The grafts in rabbits killed 3 weeks after surgery were already completely vascularized. Basal lamina grafts contain no live oxygen-requiring cells, so they do not need to be vascularized to survive; only when axons and Schwann cells enter the graft does an adequate blood supply become essential. In this respect basal lamina grafts have a theoretical advantage over nerve autografts, which are made up of live cells and require a continuing supply of oxygen and other nutrients. Nerve autografting involves disconnecting the graft from its blood supply, which means that cells in the graft center will probably die soon after operation. For nerve autografts to work properly the Schwann cells probably need to be alive, since clinical and experimental experience with frozen nerve grafts involving dead Schwann cells has been generally unsatisfactory. 12,19,26,29

5. Preserving the topographical relationships of the axons in a repaired nerve may help to ensure that regenerating nerve fibers reach their correct targets. 4,5,10 Reconnecting individual fascicles is one way to preserve the internal organization of the nerve, and basal lamina grafts could be used for this purpose. It may also be of some importance that regenerating axons preserve their interrelationships by growing parallel to one another within the graft. Muscle basal lamina grafts are composed of parallel tubes of basal lamina, and the axons grow within these tubes. Attaching the two ends of the graft correctly, therefore, will ensure that the regenerating axons grow parallel to one another in the graft.

Grafts made from basal lamina tubes derived from muscle seemed to be as good as nerve autografts for peripheral nerve repair in our experiments. They have a number of advantages: they can be made in whatever shape and size is needed; they can be stored indefinitely; they do not have to be surgically removed from a large incision in the leg; and they can probably be made from animal material. It remains to improve the methods of graft preparation, and to see whether basal lamina grafts work in animals such as monkeys, whose powers of nerve regeneration more closely match those of humans.

Acknowledgments

We thank Jim Rokos and Steve Pfeffer for their expert technical assistance, and Dr. W. M. Cowan for his support and comments on the manuscript.

References

15. McCollester DL: A method for isolating skeletal-muscle...
Muscle basal lamina graft for nerve repair


Manuscript received October 30, 1985. Accepted in final form February 10, 1986. Dr. Keynes was the recipient of a Wellcome Trust travel grant while working on this project.

Present address of Dr. Keynes: Department of Anatomy, Cambridge University, Downing Street, Cambridge CB2 3DY, England.

Address reprint requests to: James W. Fawcett, M.B.,B.S., Ph.D., M.R.C.P., The Salk Institute, P.O. Box 85800, San Diego, California 92138.