Experimental Studies on Peripheral Nerve Repair: 
a possibility of application to cure nerve 
complication of Hansen’s disease

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[Received 1 Jun 1999]

Key Words: peripheral nerve, repair, Hansen disease

I) Background:

When a nerve cell survives after a trauma, numerous very thin axons actively sprout from the central portion of the cut ends of the axons. When the tissue conditions are suitable reinnervation occurs. The purpose of most experimental and surgical procedures is to manipulate how the sprouting effectively grows and to reinnervate the peripheral effector tissue.

In pathological diagnostic routine work we have very often seen findings from muscle biopsies, which have been mentioned in many studies, of de- and reinnervation in the muscle tissue. We are interested in this pathogenesis, and ascertaining morphologically de- and reinnervation of muscle fibers over a time course. For peripheral nerve repair there have been numerous papers on human and animal experiments. Among these, other than mechanisms, all are seeking technical methods to get better repair results. Microsurgical manipulations of cut ends, and nerve grafts which are the best recent methods still pose many difficulties in humans and in experiments. There are many papers concerning various kinds of tubulation methods instead of microsurgery and grafts. According to a review by Suematsu [1], since Glueck has used decalcified bone for tubulation, various kinds of material have been used. These have included collagen tubes, silk thread bundles, silicone tubes, mesothelial tubes, millipore, amnioplastin, arteries, veins, muscles, fascial tubes, perineurium, and synthetic microporous tubes. Marshall et al.[2] utilized resorbable polyglycolic acid. Takahashi[3], Rosen[4] and Hosokawa[5] presented papers utilizing a collagen membrane, which is bioresorbable in tissue, in order to wrap cut ends of the nerve.

The method using silicone tubes is also useful for not only easy bridging, but also research on various growth factors[6] and materials[7] in the tube in order to accelerate axon sprouting over long distances[8].

Among the experimental models utilizing such varied materials, because of the easy manipulation of the cut ends, and its high success in reinnervation, we employed a tubulation method utilizing silicone tubes. Using silicone tubes with some modifications we collected electrophysiological, morphological histochemical, and
morphometrical research data on the regeneration of sciatic nerves in the tube, muscle branch nerves, intramuscular nerves and end plates\textsuperscript{3,5,9-12}.

The present paper briefly describes, reviewing the basic results of our data on successful regeneration and reinnervation using the tubulation method with silicone tubes, and mentions its clinical use for not only traumatic human cases, but also the possibility for its use with the severe neural complications of Hansen disease.

II) Experimental methods:

The right sciatic nerve of mature male Wistar rats was severed. The cut ends, central and peripheral sides were inserted into a silicone tube, 7 mm long and with a 1 mm inner diameter. A five mm gap was left empty between both ends. We examined histologically, immunohistochemically, and morphometrically the thickness and numbers of axons with light and electron microscopic sections. They were taken from tissue in the silicone tube, soleus muscle branch nerves, intramuscular nerves, and the end plate of the muscle fibers. A schematic picture (Fig. 1) shows the location from where tissue specimens were taken. The tissues were examined chronologically from 1 to 60 weeks, depending on the locations. For the intramuscular nerves of the soleus, we evaluated the number and thickness of axons in accordance with the thickness of nerve fascicles\textsuperscript{13}.

As shown in Fig. 2, they were classified as less than 10 (class 1), 10-20 (class 2), 20-30 (class 3), and 30-50 µm in the shorter diameter of the nerve fascicles. For most terminal nerve axons of the intramuscular nerves and end plates, the specimens were stained with both acetylcholine esterase (AchE) and Bodian silver stain. They were then used for qualitative analysis with light microscopy.

III) Results:

1. Regeneration of tissue in the tube

One week after the operation there were no axons, but massive fibrin nets were found under light microscopy in the tissue from the middle of the silicone tube. Two weeks after the operation
it was occupied by mostly Schwann cells and a few axons (Fig. 3a). The histogram showed all axons were unmyelinated, and not myelinated. Also, there were about 4 times more axons than the control sciatic nerve. These axons did not show bimodal distribution of axon thickness, unlike the control histogram. About 5 weeks after the operation, the proximal and distal stumps were united by the regenerated nerve trunks in the silicone tube. Microscopically, numerous axons were seen with Bodian stain (Fig. 3b). Histograms detected that all axons were thin and had increased in number, and myelinated axons were found to be about 4 times more in number than the control sciatic nerve. Also, unmyelinated axons at this time were thin and had increased to about 5 times the number in the control.

2. Soleus muscle branch of the tibial nerve:
One week after the operation, the soleus muscle branch nerve showed neither myelinated nor unmyelinated axons, but mostly macrophages by light and electronmicroscopy. Six weeks after the operation, few nerve fibers with very thin myelin sheaths, were found along with macrophages and Schwann cells. The proliferation of nerve fibers formed a compartmentation after about 12 weeks, and thicker axons co-existed thereafter. After about 9 months from the operation, numerable regenerated axons were found and they had almost the same characteristics as the regenerated sciatic nerve in the silicone tube.

Sixty weeks after the operation, it was similar to the control. However, axons were morphometrically about 64% the mean diameter, and 187% in number compared with the control branch nerve.

3. Intramuscular nerves of the soleus muscle:
From 1 to 2 weeks after the operation, the structure of the intrafascicular nerve fibers was broken. Disintegration of the myelin sheath, myelin debris, and macrophages were observed. The nerve terminals of the neuromuscular junctions had disappeared, and clefts of the end plate were flattened and simplified seen by electronmicroscopy. Eight weeks after the operation, in a few thicker nerve fascicles (class 4, proximal), small regenerated nerve fibers began to be observed, but not in thin fascicle (class 1 and 2, distal). From 12 to 60 weeks, thicker nerve fibers began to appear, even in the thin fascicles. Also, complete end plates were recognized.

4. Nerve terminal and end plate light microscopically examined with AchE-Bodian stain:
One week after the operation, the AchE reaction of the end plates significantly decreased in intensity. It disappeared in all tissue section fields (Fig. 4a). But, 6 weeks after the operation, a few axons began to appear, and the AchE reaction reappeared after about 8 weeks. Collateral pre-terminal sprouting and polyinnervation (Fig. 4b) increased the most after 16 weeks. They were 36.22% as a proportion of all end plates, and this gradually decreased to 12.36% 60 weeks after the operation.

Fig. 3. Bodian stain of a section from the mid portion in a tube reveals axons in (a) and (b), at the time of 2 and 5 weeks, respectively after the operation. A few of axons among Schwann cell nuclei in (a) and innumerable in (b).
IV) Discussion and clinical application:

The aim of most similar research has been into better regeneration and reinnervation in clinical and experimental settings. Nerve axons have a very large potential for the regeneration of axons, but manipulation is also important to achieve a high success rate. From our results, the silicone tube method is the easiest and has the highest success rate. Also, our results indicated that in the substance in the tubes, collagen matrix, and material of the tube itself, resorbable collagen film was related to the speed of axon sprouting and the thickness of the regenerated axon diameters. Also, the results indicate that in the most peripheral region, nerve terminals and end plates in our experiments, there was no significant difference between the tube material, and the substance in the tube. As for the most peripheral regions, it was suggested that the most dependent factor might be the interaction between regenerating nerve terminals and end plates. There are studies\textsuperscript{14,15} suggesting the ability for selectivity of motor and sensory nerves in reinnervation. The exact mechanism has not been elucidated, but we consider simple manipulation by inserting cut ends into silicone tubes may result in selective reinnervation of both sensory and motor terminals.

For the clinical use of silicone tubes G. Lundborg et al.\textsuperscript{16} compared the method with the microsurgery technique on median and ulnar nerve injury patients (11 cases with silicone tubes, 7 cases for microsurgery). After a 1 year follow up, the patients recovered equally well in the sensory and motor hand functions. As for Hansen disease cases, we read a few papers on surgical treatments of peripheral disturbances in patients. Pereira et al.\textsuperscript{17} operated on the posterior tibial, or median nerves of a total 10 patients. The duration of the disease was from 4-12 years. They all suffered from sensory disturbances for 1.5-7 years. Autologous sartorius muscle tissues which were denatured by a freeze were grafted between a 2.5-6 mm gap, and the follow up was done for 8 to 18 months. Vibration, joint position sense, perception of a 10 gm pin, and ability to sweat in the affected area was restored in 7 patients.

V) Conclusion

Pereira report was amazing because in spite of suffering from nerve disturbances for up to 7 years, sensory nerves showed the ability to recover.

In our opinion, the manipulation of silicone tubulation is technically easier, simpler, more efficient and safer than other available methods. We emphasize that with effort, trial and improvement in the application of tubulation, silicone tubes may be a very valuable surgical tool to cure Hansen disease patients from peripheral nervous disturbances that are their most major complications.
References


末梢神経修復の実験的研究：
ハンセン病末梢神経障害治療への応用の可能性

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【受付：1999年6月1日】

キーワード：末梢神経、修復、ハンセン病

神経細胞が生きている限り、神経線維が切断されても中枢断端からsproutingを極めて活発に芽生えるので、切断部分での適切な処置。そして末梢効果器に機能が保存されている条件下にて、末梢神経線維の再接続が達せられるならば、形態的、機能的修復が可能である。ほとんどの実験的、臨床的努力は切断部分の処置による有効なsproutingの芽生え末梢への再支配を目的としたものである。その関係の論文も極めて多い。このうち移植を含めた外科的方法は現実臨床の主流であるが、多くの問題点を伴っている。これに替わる方法として実験的には切断端をtubeにて包むtubulationが盛んである。tubeの材料もきわめて種々のものが古くから試みられてきている。幾つかの理由により我々はsilicone tubeを用いることに基本をおき、筋細胞への神経再支配を検証してきた。その臨床応用の実際については、ごく最近にいたり、末梢神経損傷患者にsilicone tubeを用い、従来のmicrosurgeryの方法と比較して遜色ない結果がLundborgら(1997)されている。一方で、ハンセン病患者の末梢神経障害に対する外科的治療はほとんど行われていない現状のようである。しかしJ.H.Pereiraら(1991)は1.5から7年間の知覚障害をもった10例の患者に対し、神経断端に水結性変性をきたした自家縫合筋移植を行って7例の患者において手足の知覚や発汗の回復を得た報告している。このことは筋移植より容易に操作が可能なsilicone tubeをハンセン病患者に応用できる可能性を示唆している。本reviewでは神経再支配の所見とハンセン病患者の臨床応用の可能性について述べた。

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