Nerve synthesis in vivo (regeneration)*

1. Anatomy and function of a peripheral nerve.
2. Experimental parameters for study of induced regeneration.
3. Synthesis of myelinated axons and BM (nerve fibers)
4. Evidence (?) of synthesis of an endoneurium.
5. Synthesis of a nerve trunk (including summary of kinetics of synthesis).
6. Comparative regenerative activity of various reactants.

******Tissue and Organ Regeneration in Adults***, Yannas, Springer, 2001, Ch. 6.**

1. Anatomy and function of a peripheral nerve. I

Nervous system = central nervous system (CNS) + peripheral nervous system (PNS)

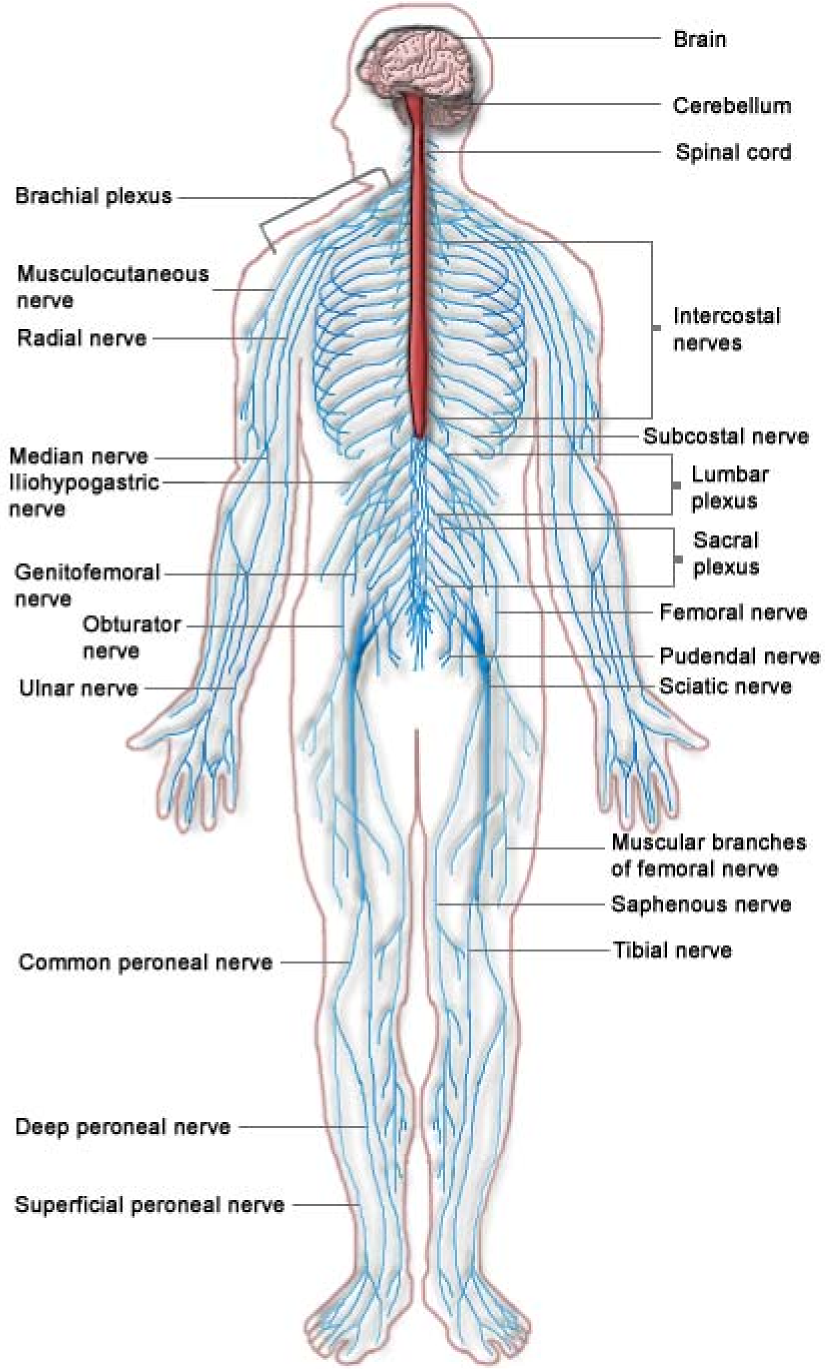
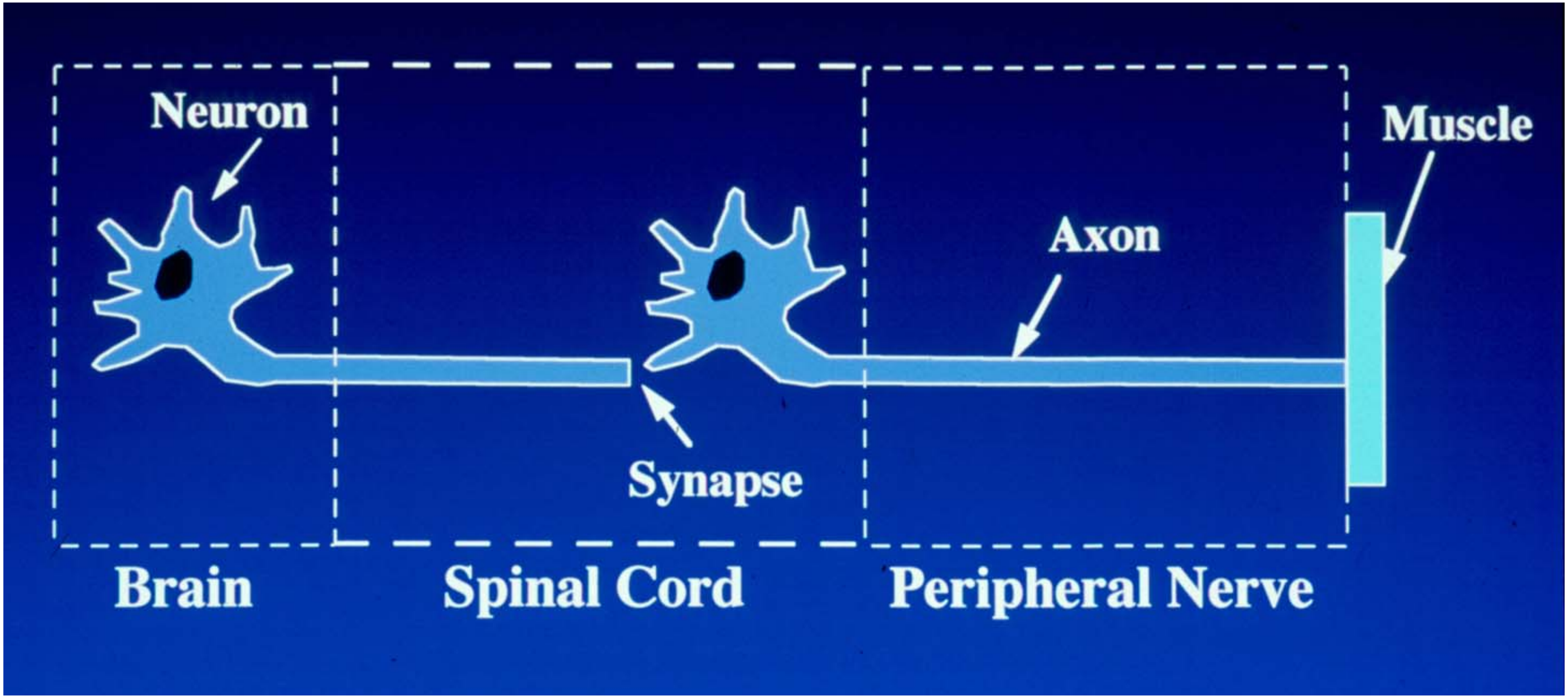


Image: public domain (by Wikipedia User: Persion Poet Gal)

Nervous System: CNS and PNS



CNS **PNS**

Chamberlain, Yannas, et al., 1998

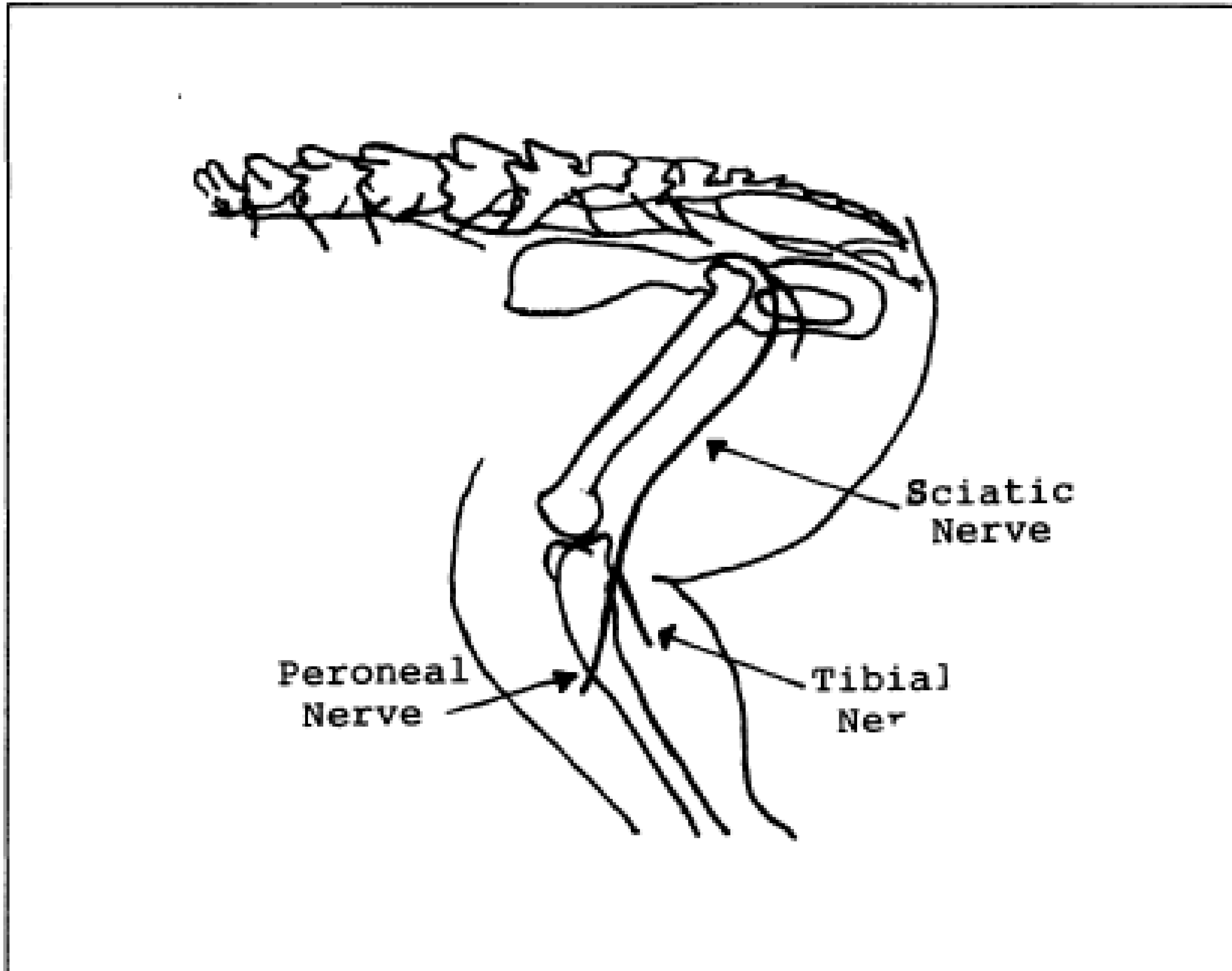


Figure 2-1: Rat hindquarter, showing location of sciatic nerve.

Landstrom, Aria. "Nerve Regeneration Induced by Collagen-GAG Matrix in Collagen Tubes." MS Thesis, MIT, 1994.

Focus of interest: nerve fibers and axons

Nerve fibers comprise axons wrapped in a myelin sheath, itself surrounded by BM (diam. 10-30 μm in rat sciatic nerve).

Axons are extensions (long processes) of neurons located in spinal cord. They comprise endoplasmic reticulum and microtubules.

1. Anatomy and function of a peripheral nerve. II

Myelinated axons (diam. 1-15 μm) are wrapped in a myelin sheath; nonmyelinated axons also exist. They are the elementary units for conduction of electric signals in the body. Myelin formed by wrapping a Schwann cell membrane many times around axon perimeter. No ECM inside nerve fibers.

Myelin sheath is a wrapping of Schwann cell membranes around certain axons.

1. Anatomy and function of a peripheral nerve. III

Nonmyelinated axons (diam. <1 μm) function in small pain nerves. Although surrounded by Schwann cells, they lack myelin sheath; Schwann cells are around them but have retained their cytoplasm.

Basement membrane (tubular) encases the myelin sheath. Structure similar to that of skin BM.

1. Anatomy and function of a peripheral nerve. IV

- **Nerve fibers are embedded in <u>endoneurium</u>: a delicate packing of loose vascular supporting tissue that is rich in collagen fibers. Definitely ECM!**
- **Many nerve fibers with their associated endoneurium are packed in a collagenous layer, the <u>perineurium</u>. This forms a <u>fascicle</u>.**
- **Multifascicular nerves encased in a collagenous layer, the <u>epineurium</u>.**

Cylindrical symmetry of peripheral nerve structure

<u>Summary of nerve trunk structure</u>

proceeding <u>radially</u> from the center:

[axon — myelin sheath — BM] — endoneurium — perineurium — epineurium.

[….] = “nerve fiber”

Cross section of rat sciatic nerve ("nerve trunk").

Several thousand nerve fibers.

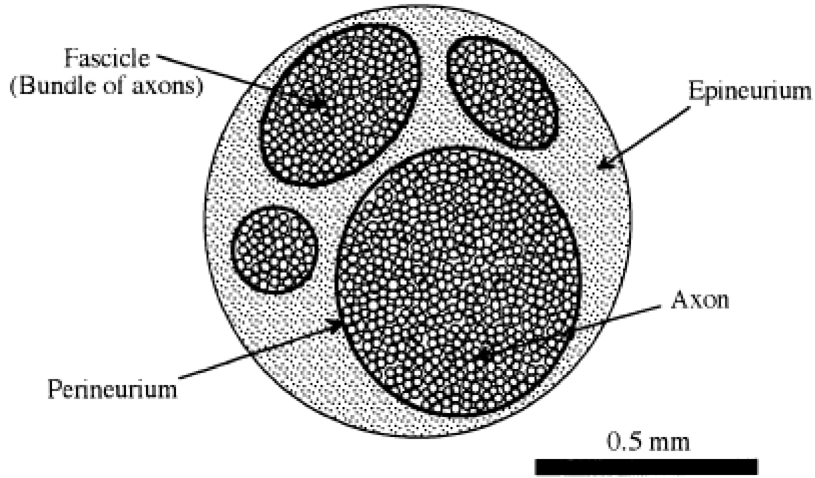
Noncircular cross section.

Jenqa, C. B., and R. E. Coggeshall. *Brain Research* 326, no. 1 (1985): 27-40.
Courtesy of Elsevier, Inc., http://www.sciencedirect.com. Used with permission.

(idealized)

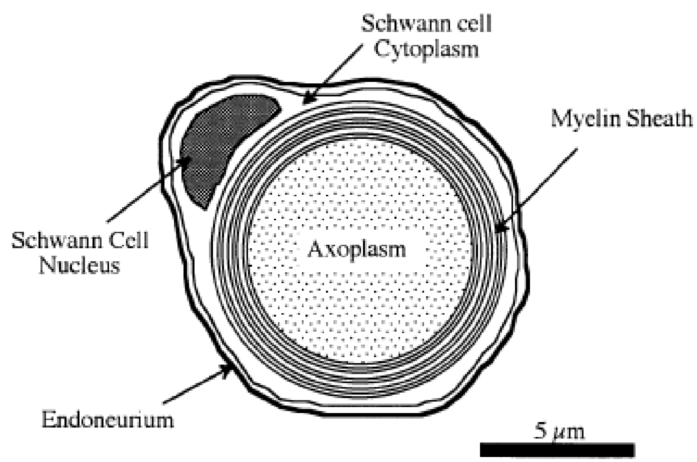
Rat sciatic nerve cross section

nerve trunk



Individual axon

nerve fiber



Chamberlain, L. J. "Long Term Functional and Morphological Evaluation of Peripheral Nerves Regenerated Through Degradeable Collagen Implants." MIT M.S. Thesis, 1994.

Longitudinal view of nerve fiber

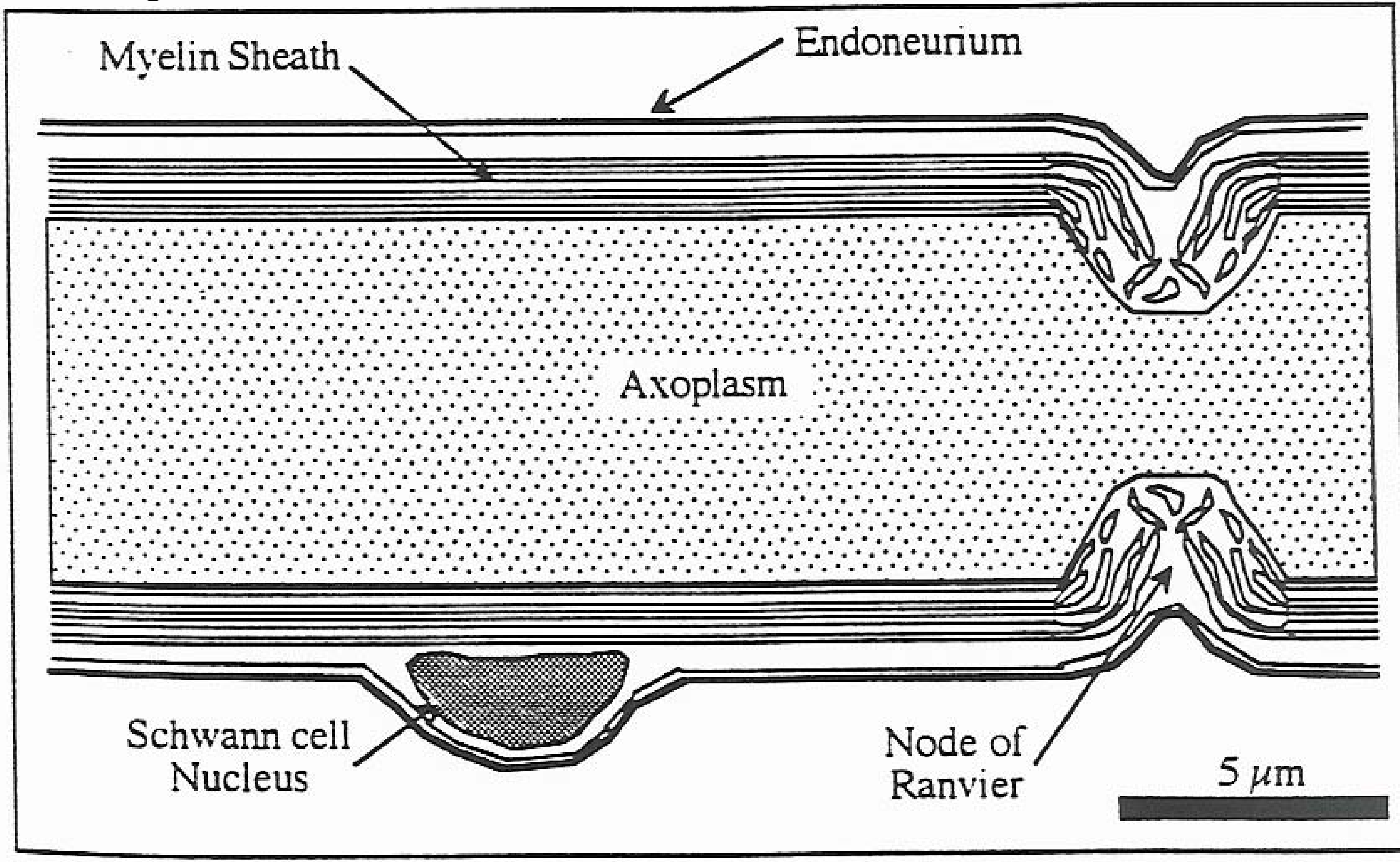


FIGURE 1.3 Schematic showing a longitudinal section of a normal myelinated axon.

Chamberlain, L. J. "Long Term Functional and Morphological Evaluation of Peripheral Nerves Regenerated Through Degradeable Collagen Implants." MIT M.S. Thesis, 1994.

Myelination of a nerve fiber during development or during induced regeneration

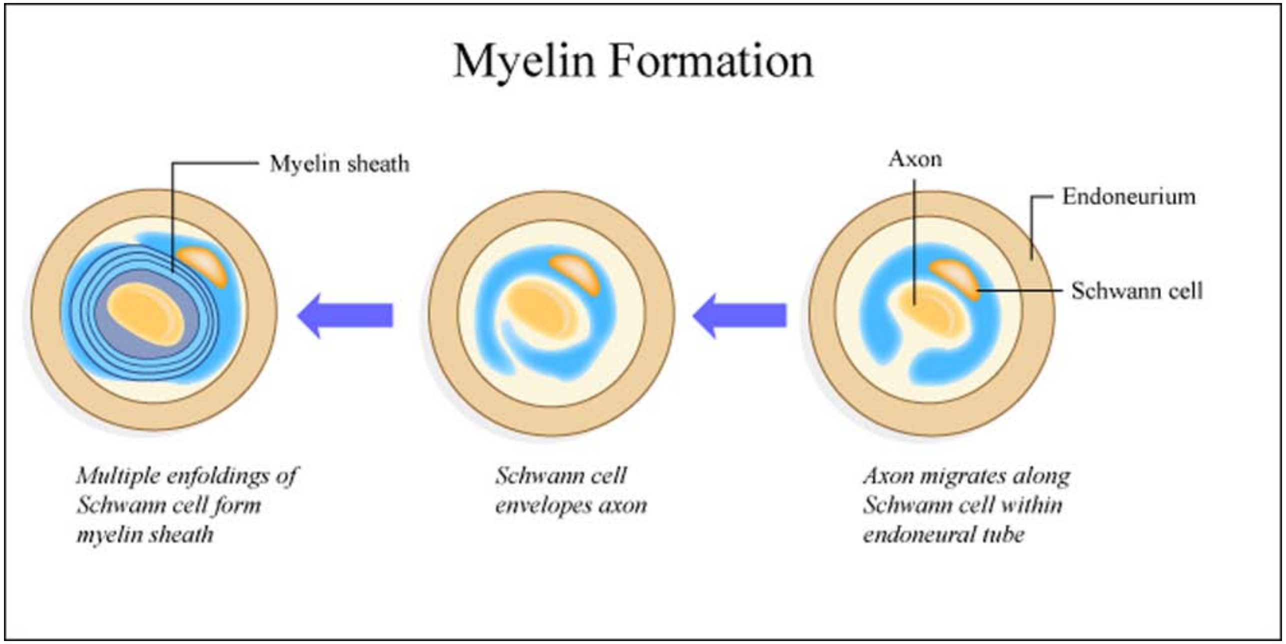


Figure by MIT OpenCourseWare.

2. Experimental parameters for study of regeneration

A. <u>Anatomically well-defined defect</u>

– Designate experimental volume

– Delete nonregenerative tissue(s)

– Anatomical bounds

– Containment of exudate

B. <u>Timescale of observations</u>

– Short-term (<20 wk) and long-term (>20 wk) assays

Regenerative similarity of tissues in skin and nerves. Identify epithelial tissue, BM and stroma.

	Skin	Peripheral nerves
Regenerative Tissues	Epidermis Basement membrane	Myelin sheath Basement membrane (perineurium, in part only)
Nonregenerative Tissues	Dermis	Endoneurial stroma

The injured myelin sheath regenerates spontaneously

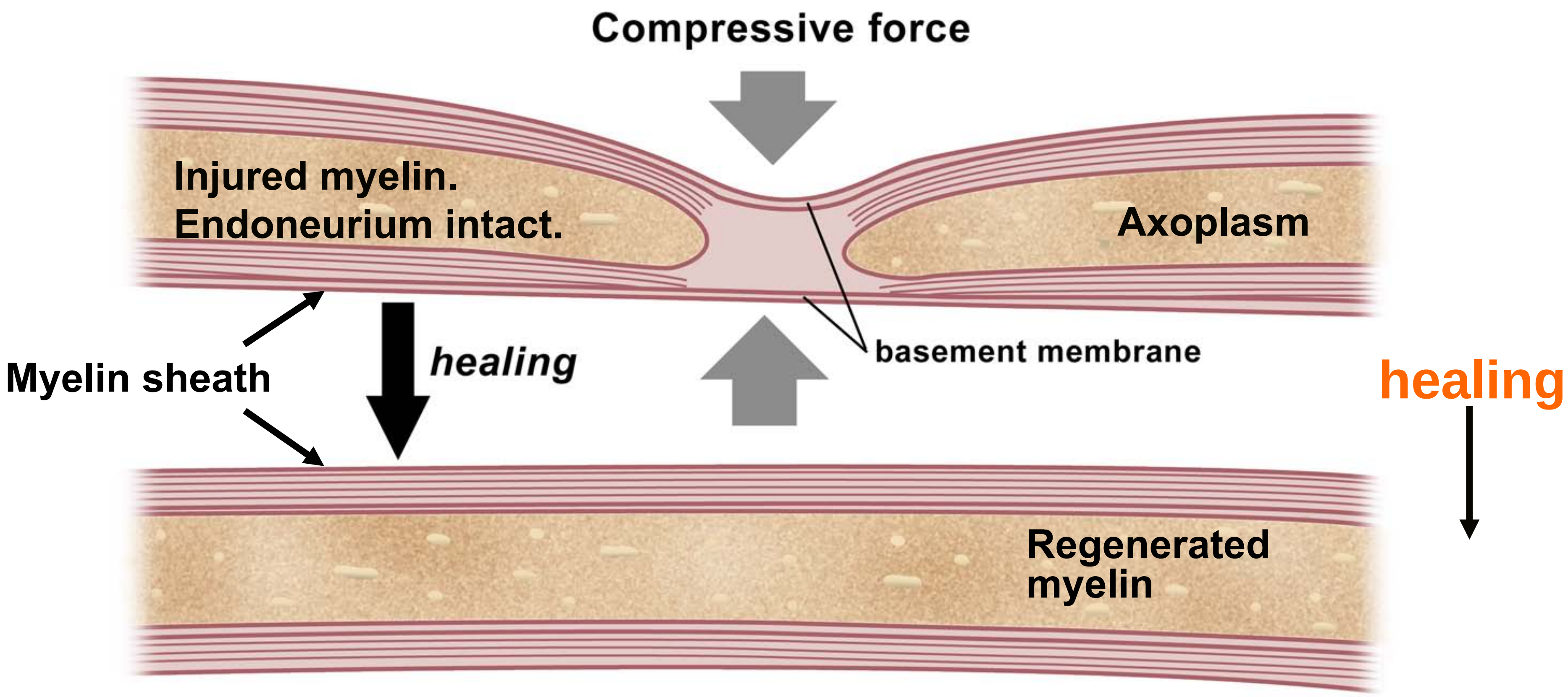


Figure by MIT OpenCourseWare.

Neuroma formation. The endoneurium does not regenerate. Contraction and scar formation.

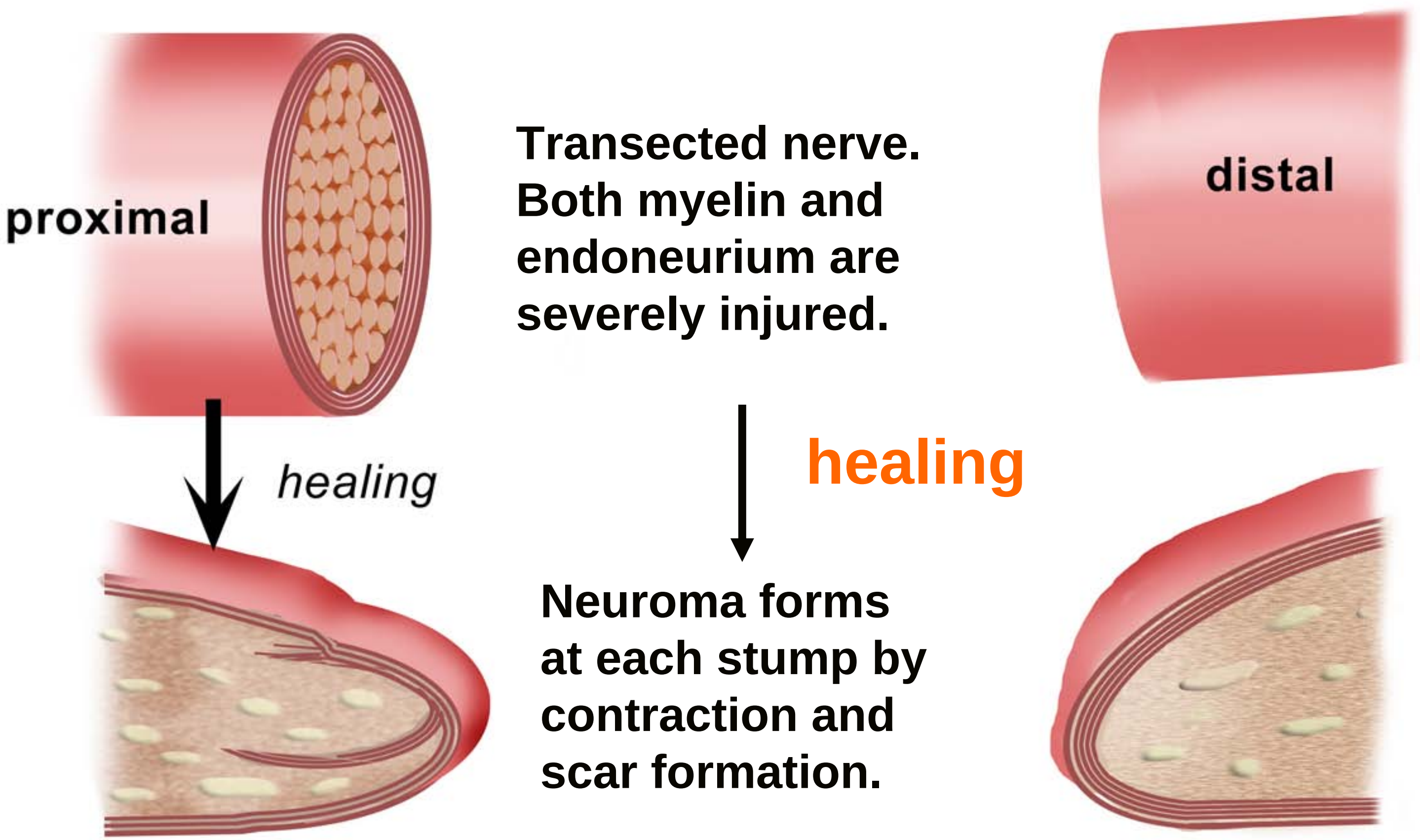


Figure by MIT OpenCourseWare.

Intact nerve fiber with myelin sheath (left, black margin) and associated Schwann cell (right). Endoneurium outside.

Histology photo of nerve fiber removed due to copyright restrictions.
See Figure 2.5 (top) in [TORA].

Healing following transection

Spontaneously healed nerve fiber filled with scar (Büngner bands, Bb)

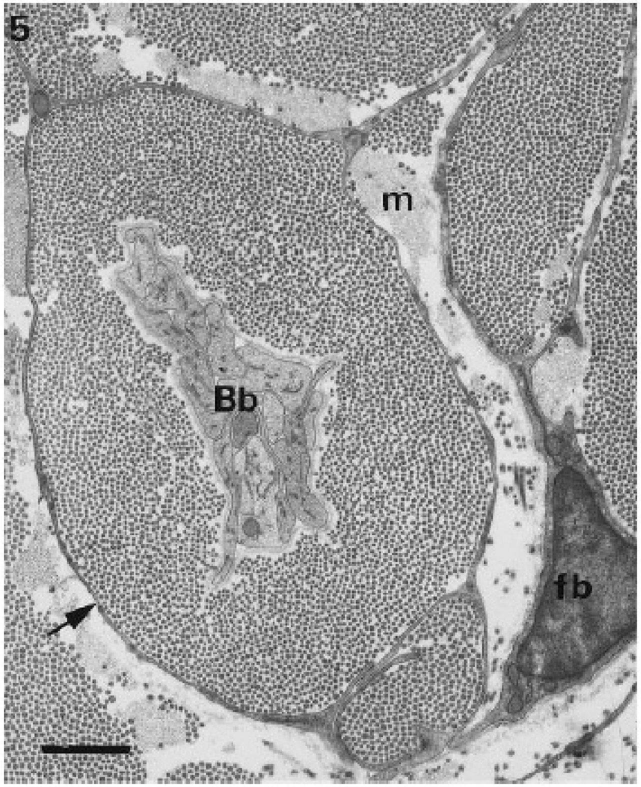


Fig. 5. Electron micrograph of a collagen domain containing a central Büngner band (Bb). The domain is encircled by thin fibroblast processes (arrow) which interdigitate in the upper right of the figure. These processes do not posses a basal laminal ensheathment whereas the fibroblast (fb) in the lower part of the figure shows definite perineurial transformation, possessing patchy basal lamina and displaying multiple pinocytotic vesicles in its processes. m, microfibrils. Bar, 1 µm.

Bradley, J. L., et al. *J Anat* 192, no. 4 (1998): 529-538.

2. Experimental parameters (cont.)

C. <u>Assays of outcome</u>

– Correction for experimental gap length.

– Correction for animal species.

– Critical axon elongation, L_c.

– Shift length, ΔL. Characterization of devices.

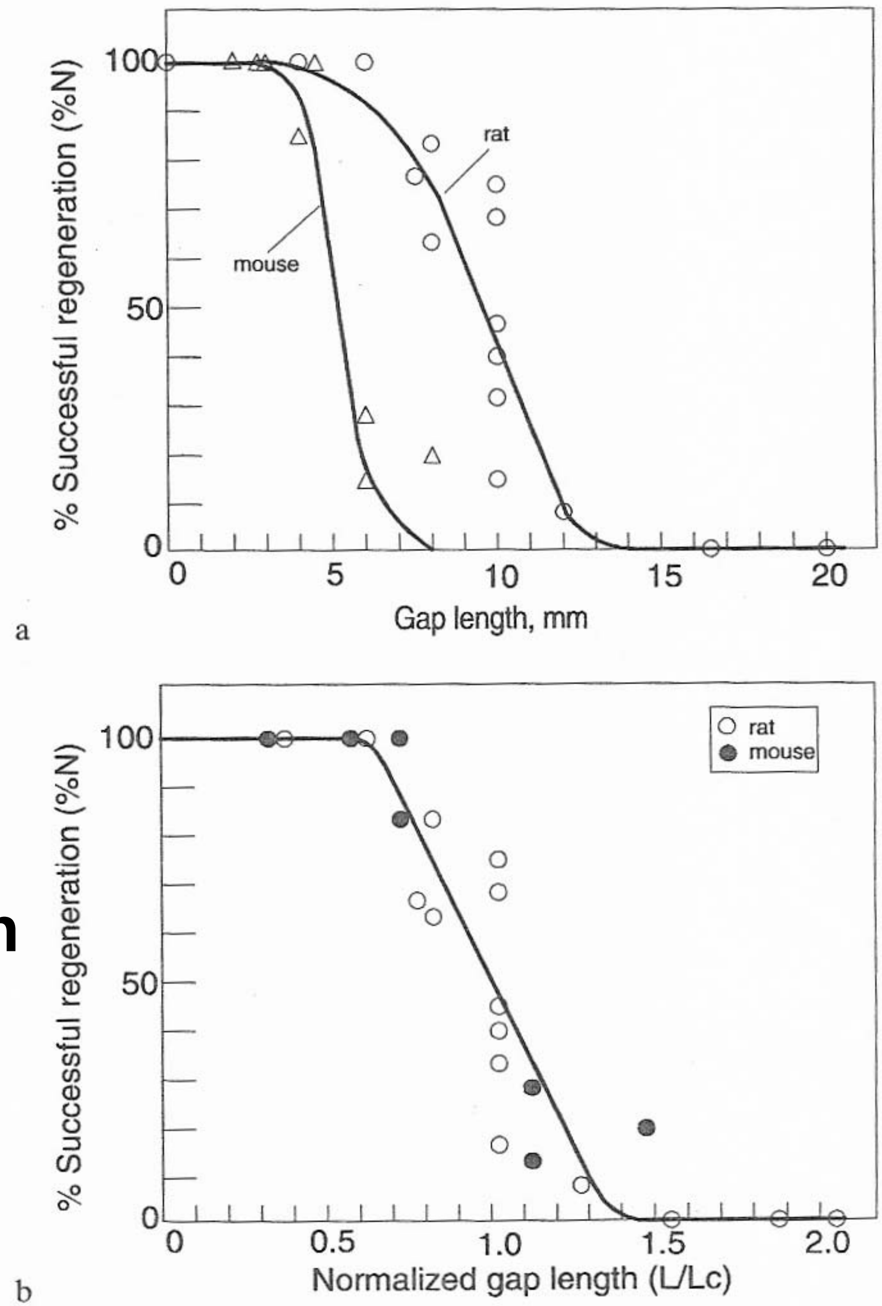
– Long-term: fidelity of regeneration.

C. Assays of outcome (cont.)

Use corrected values of frequency of reinnervation (%N) across tubulated gaps. This correction allows comparison of %N data from studies with different gap lengths and different species.

Critical axon elongation, L_c, the gap length above which %N drops below 50% (or the gap length where the odds of rein-nervation are even). Data from several investigators have shown that $L_c = 9.7 \pm 1.8$ mm for the rat sciatic nerve and 5.4 ± 1.0 mm for the mouse sciatic nerve.

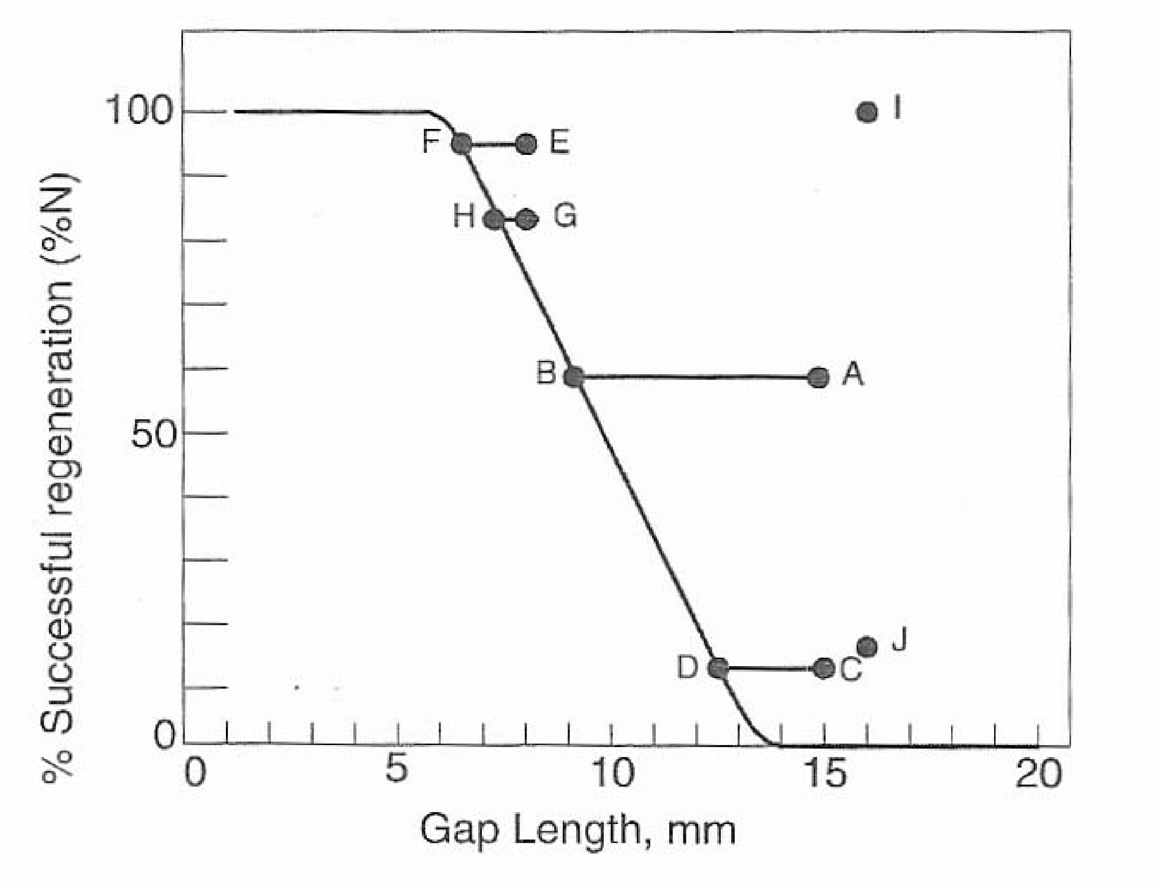
Characteristic curve defines critical axon elongation, L_c, at %N = 50%



L_c = 9.7 ± 1.8 mm for the rat sciatic nerve and 5.4 ± 1.0 mm for the mouse sciatic nerve

Data from rat and mouse superpose when plotted vs. reduced length, L/L_c

Use single data point to determine L_c for unknown device



See Appendix in [TORA].

Relation between L_c, ΔL and C, S, R terms in defect closure rule

For nerve regeneration:

Configuration	Extent of defect closure by each closure mode: % Contraction	% Scar	% Regeneration	Critical axon elongation, L_c, mm	Length shift, ΔL, mm
No tube	95	5	0	≤ 6.0	≤ -2.0
Silicone tube	53	0	47	8.0	0
Collagen tube	0	0	100	≥ 13.4	≥ 5.4

Data is from three experiments using tubes filled with PBS to bridge 10-mm gap in rat sciatic nerve (estimates based on data from Chamberlain, Yannas, Hsu, and Spector. *J. Comp Neurol*. 417 (2000): 415-430.)

2A. Synthesis of myelinated axons

[NB: <u>Neuron in culture</u> provides spontaneous outgrowth of axons that serve as “substrate” for synthesis of myelin and BM. Schwann cells also obtained in culture from a neuron.]

A <u>myelin sheath</u> around axons has been synthesized in vitro in the presence of Schwann cells, with or without presence of an ECM component.

2B. Synthesis of nerve BM

A BM has been synthesized in vitro in presence of <u>neurons and Schwann cells</u>.

However, <u>neurons were not required</u> to be present when fibroblasts were cultured with Schwann cells.

Even <u>fibroblasts not required</u> when laminin added to neuron-free Schwann cell culture.

3. Evidence (?) for synthesis of an endoneurium

<u>Structure</u>. Endoneurial microenvironment surrounding each nerve fiber comprises blood vessels coursing through space filled with fluid and thin collagen fibers (51-56 nm diam.). Fluid outside blood vessels is maintained under small, positive hydrostatic pressure. Endoneurial blood vessels comprise cells that are bound by tight junctions and constitute a permeability barrier.

<u>Function</u>. Endoneurial environment protects nerve fibers from changes in ionic strength and from pathogens in blood vessels that might modify conductivity (“blood-nerve barrier”).

Endoneurium

Image removed due to copyright restrictions. See Figure 6.2 in [TORA].

[TORA] = Yannas, I. V. *Tissue and Organ Regeneration in Adults*. New York, NY: Springer-Verlag, 2001. ISBN: 9780387952147.

Evidence (?) for synthesis of endoneurium (cont.)

<u>In vitro</u>. No evidence for synthesis of endoneurial stroma.

<u>In vivo</u>. Nerve trunks have been synthesized with some evidence of formation of new endoneurium (stroma). Detailed studies of endoneurium not available, not even in normal nerves. Emphasis of researchers has focused on nerve fibers.

5. Synthesis of a nerve trunk (including kinetics)

Structure. A nerve trunk comprises one or more fascicles. Each fascicle comprises several thousand nerve fibers. If monofascicular, it is covered by perineurium; if multifascicular, it is covered by epineurium. A fascicle comprises the perineurium with its bundle of thousands of nerve fibers. Some nerves comprise many fascicles, each with its own perineurial sheath; these fascicles are wrapped in a collagenous tissue, the epineurium.

Function. Conducts strong nerve signals (amplitude about 10 mV) at conduction velocity of 70 m/s. Compare speed of sound: 343 m/s in dry air.

Rat sciatic nerve model

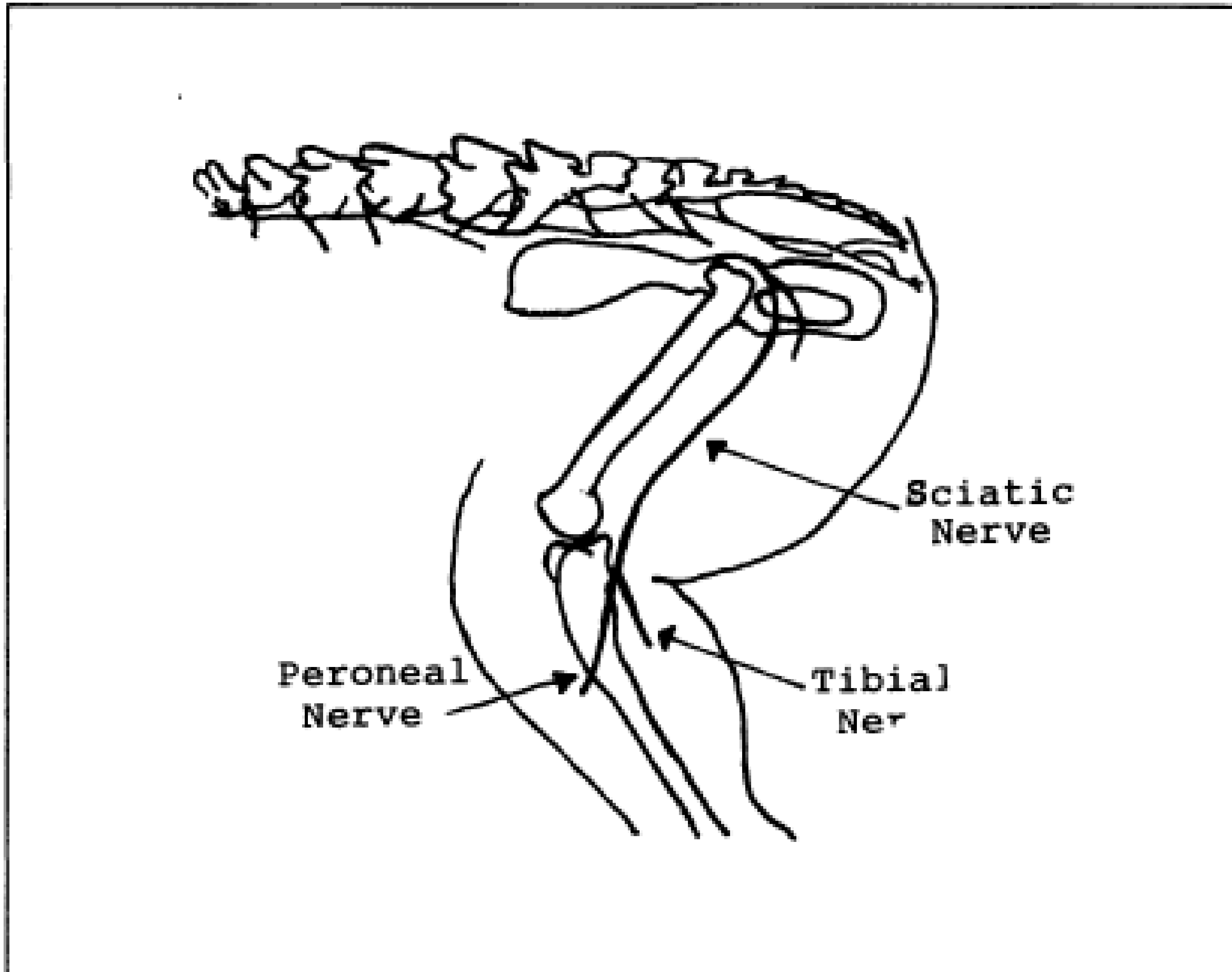


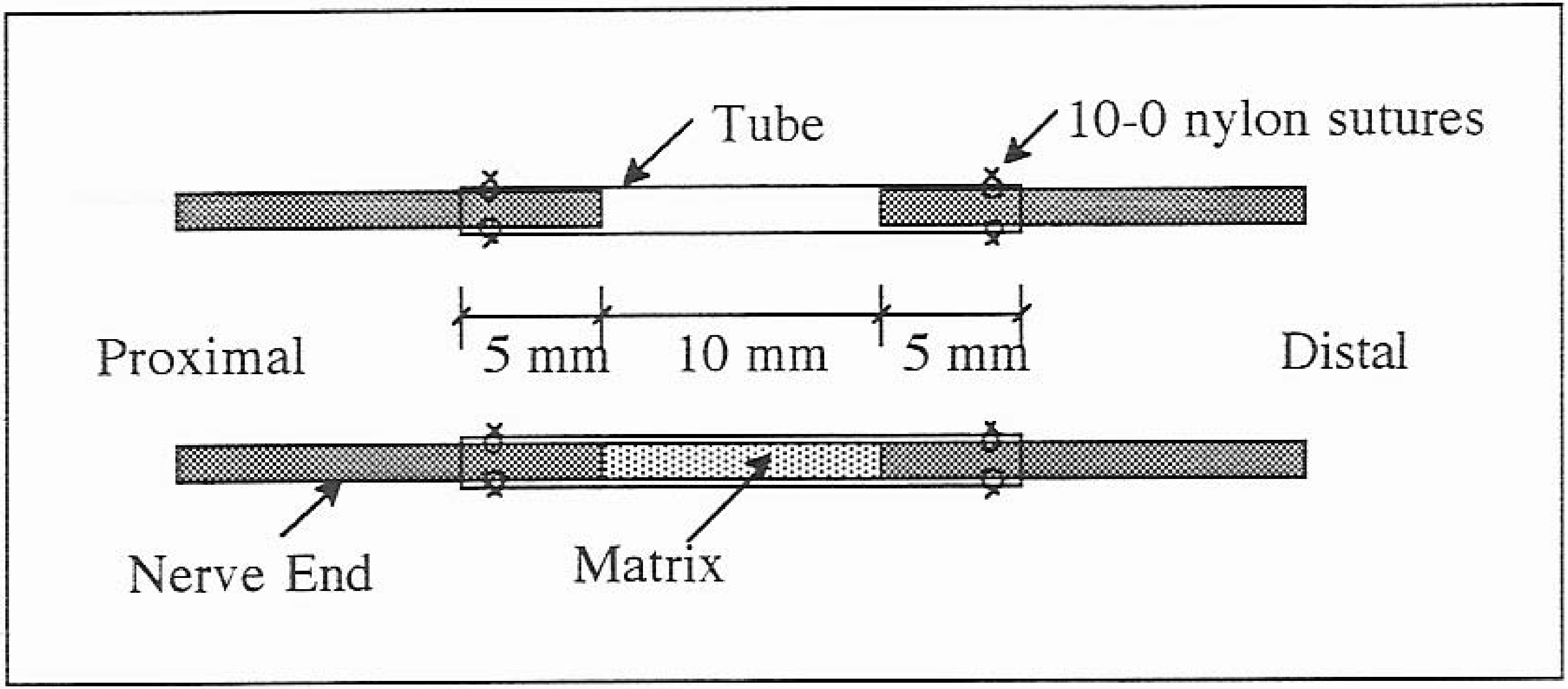
Figure 2-1: Rat hindquarter, showing location of sciatic nerve.

Landstrom, Aria. "Nerve Regeneration Induced by Collagen-GAG Matrix in Collagen Tubes." MS Thesis, MIT, 1994.

Tubulation model.

Gap length variable.

Implant Configuration

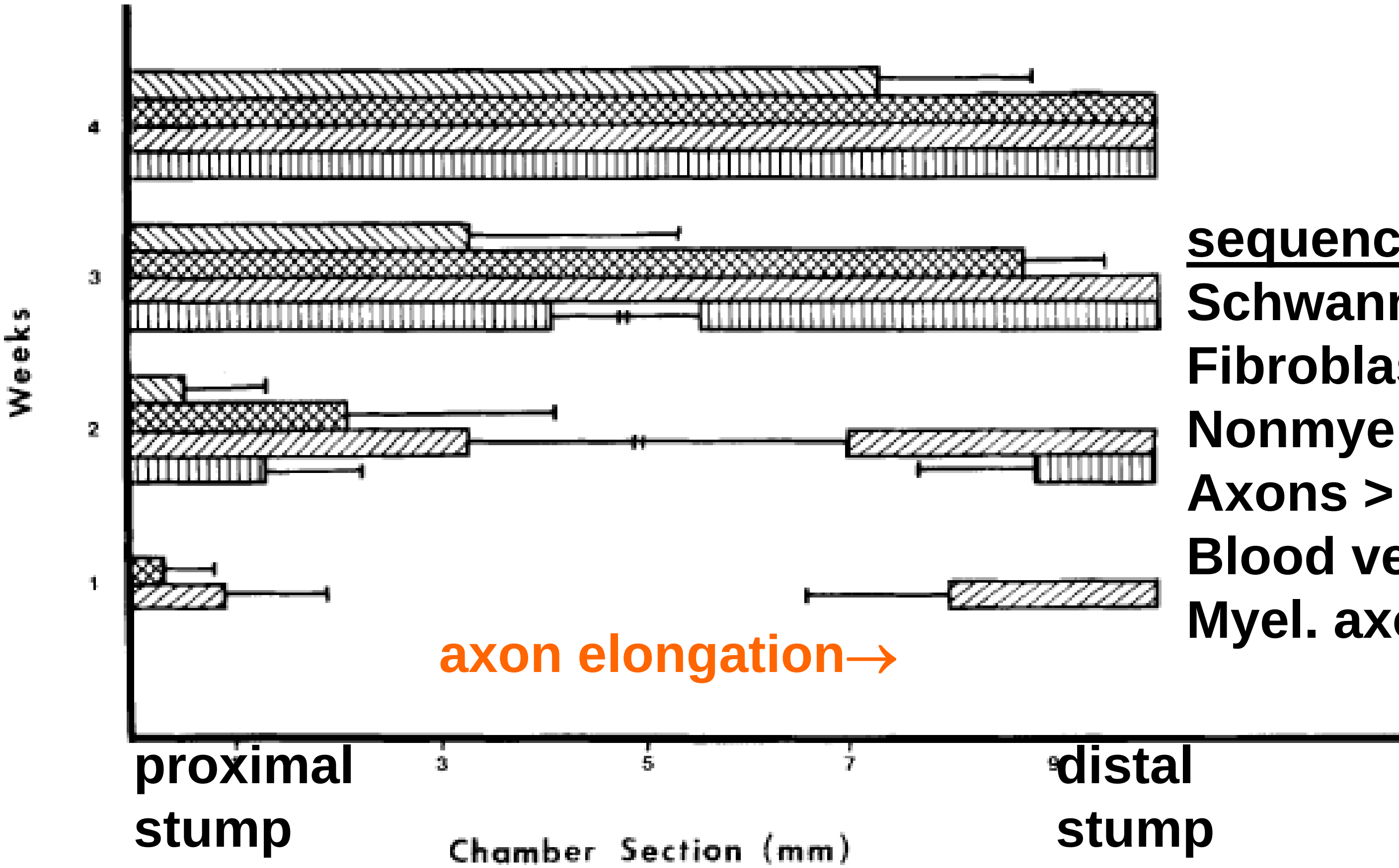


Chamberlain, L. J. "Long Term Functional and Morphological Evaluation of Peripheral Nerves Regenerated Through Degradeable Collagen Implants." MIT M.S. Thesis, 1994.

Kinetics of induced nerve regeneration

1. Compare regenerative <u>velocities</u> of elements of nerve fibers (measured inside tube model): Schwann cells + Fibroblasts > Nonmyel. Axons > Blood vessels > Myel. axons.
2. Long, almost linear, columns of Schwann cells form <u>ahead</u> of axons.
3. Contractile cell capsule surrounds regenerating nerve. <u>Thickness of capsule</u> around nerve regenerated using silicone tube was several times that of nerve regenerated using collagen tube.
4. Number of myelinated axons (strength of signal) <u>increased up to about 30 weeks</u> but reached an asymptote later.
5. Number of large diameter fibers (fibers larger than 6 μm that control <u>conduction velocity</u>) increased beyond 30 weeks and appeared to continue increasing beyond 60 weeks.

A look inside the gap



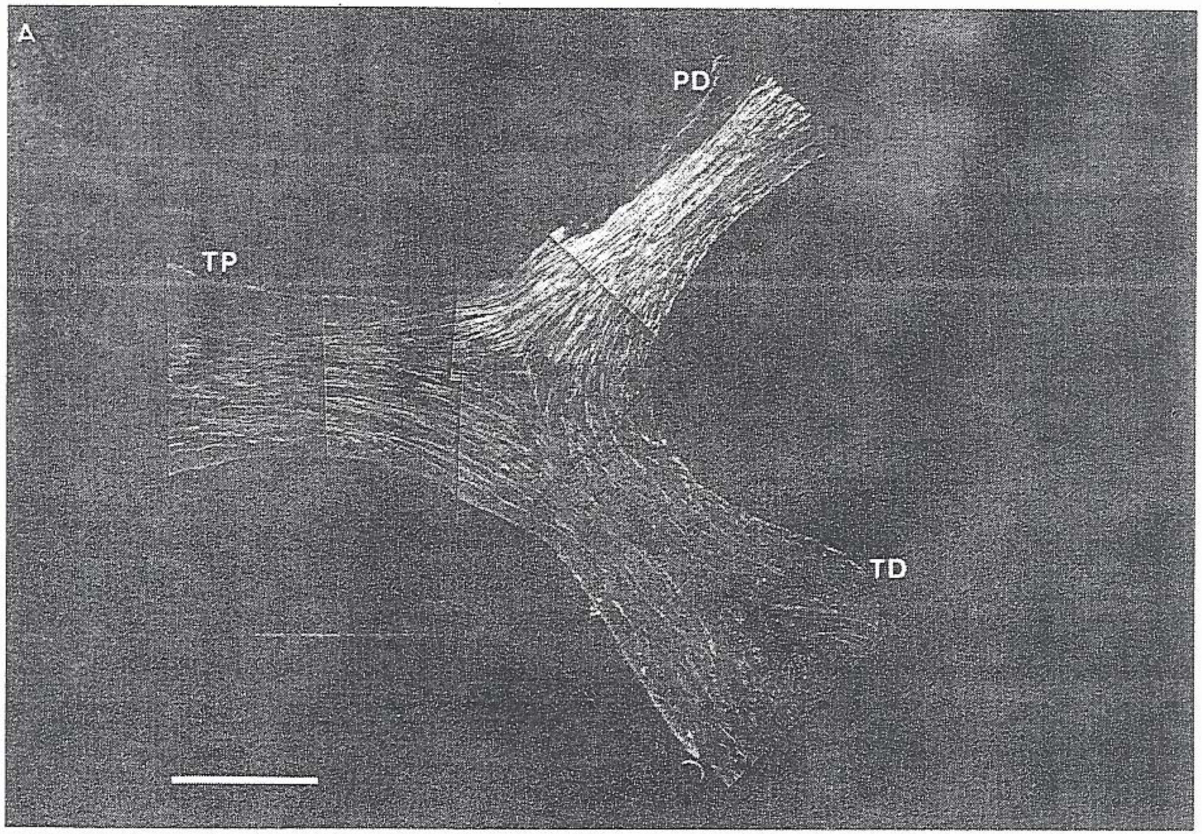
sequence:
Schwann cells + Fibroblasts > Nonmyel. Axons > Blood vessels > Myel. axons

Fig. 4. Graphic illustration of the regeneration time course. Transverse sections (S1 to S9) were scored for the presence of (1) cells (Schwann cells and fibroblasts); (2) blood vessels; (3) non-myelinated axons; and (4) myelinated axons. Averages and standard deviations were determined as described in the text.

Williams, L. R., et al. *J Comp Neurol* 218, no. 4 (1983): 460-470.

See also Fig. 10.6 and discussion in [TORA]

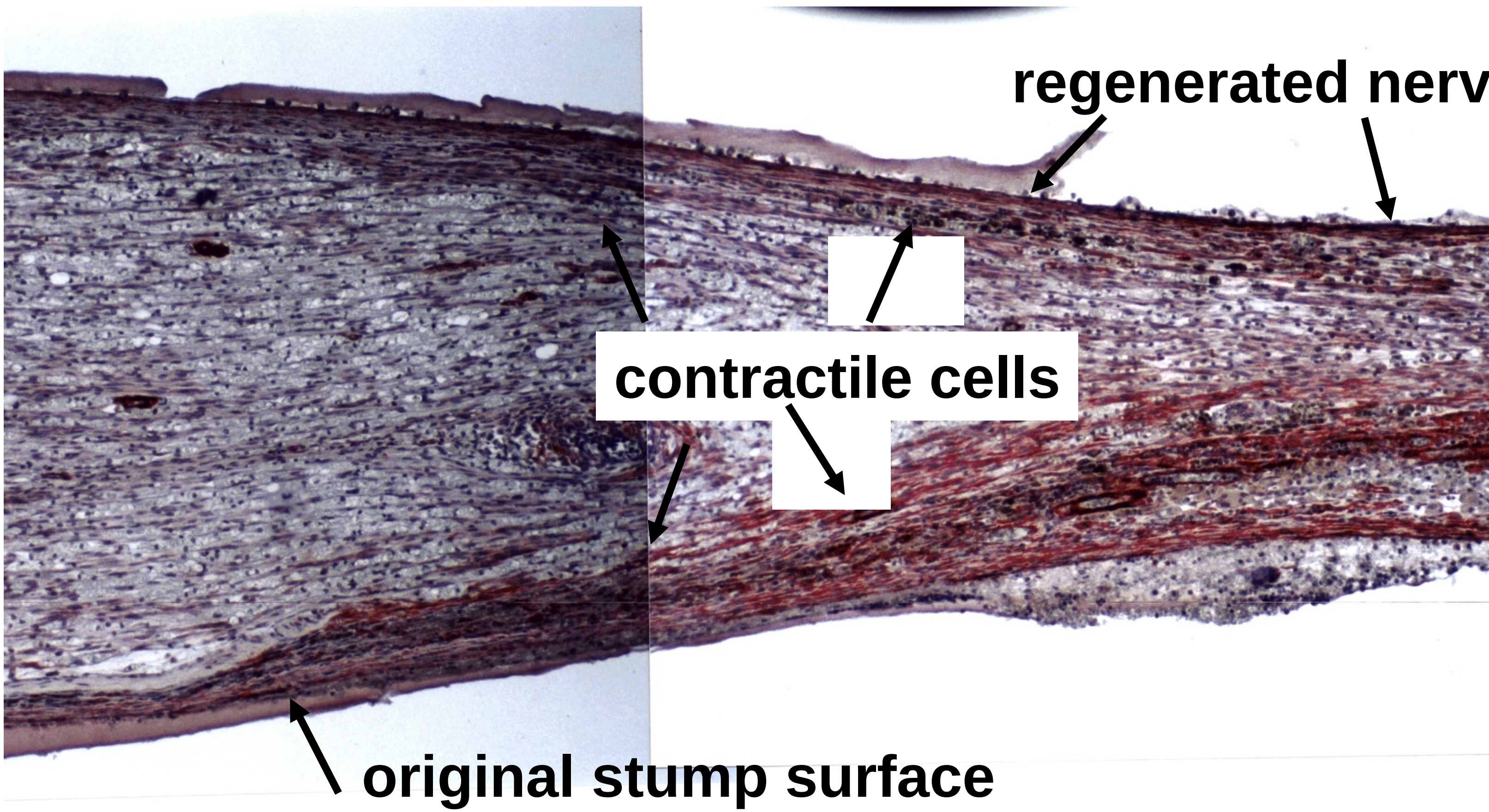
Columns of Schwann cells form even in absence of axons



Zhao, Q., et al. *Brain Research* 592, nos. 1-2 (1992): 106-114.
Courtesy of Elsevier, Inc., http://www.sciencedirect.com. Used with permission.

See also Fig. 10.8 and discussion in [TORA]

Contractile cell zone surrounds regenerating nerve



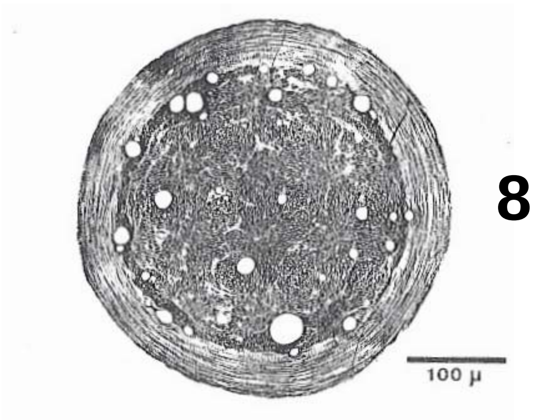
Spilker, MIT PhD Thesis, 2000

Cell capsule around regenerated nerves

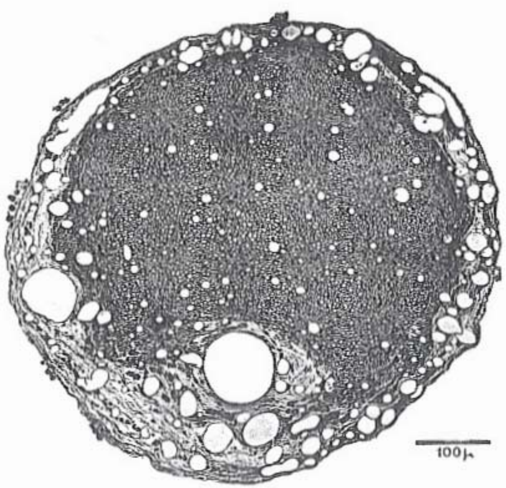
Normal rat sciatic nerve

4-mm gap

Image removed due to copyright restrictions.



8-mm gap

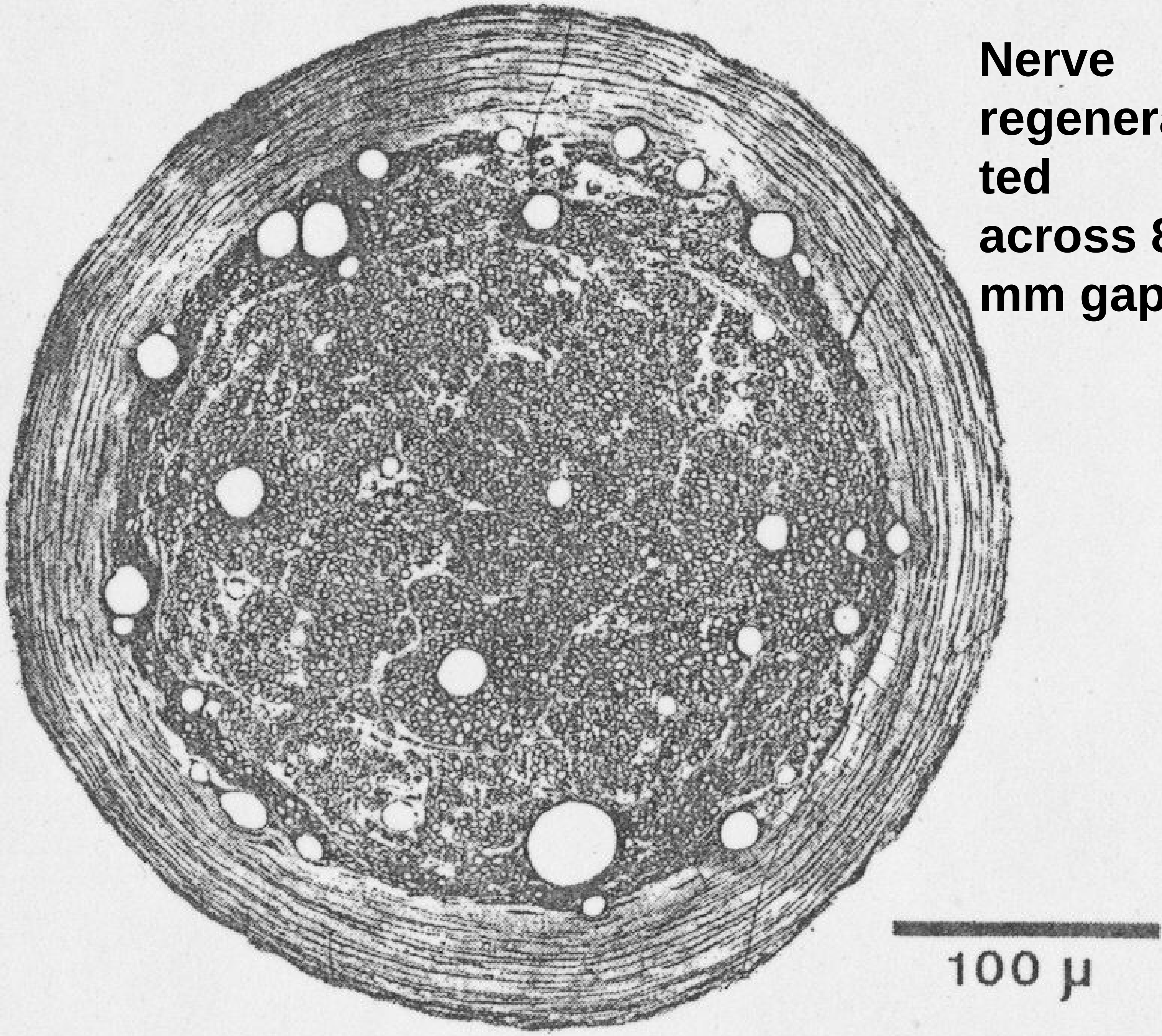


Regenerated across 0-mm gap

See also Fig. 10.7 and discussion in [TORA]

308

Nerve regenerated across 8-mm gap



Jenqa, C. B., and R. E. Coggeshall. *Brain Research* 326, no. 1 (1985): 27-40.

article by Chamberlain et al. handed out

EXPERIMENTAL NEUROLOGY **154**, 315–329 (1998)
ARTICLE NO. EN986955

Collagen-GAG Substrate Enhances the Quality of Nerve Regeneration through Collagen Tubes up to Level of Autograft

L. J. Chamberlain,* I. V. Yannas,* H-P. Hsu,† G. Strichartz,‡ and M. Spector†

**Department of Mechanical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139; †Rehabilitation Engineering R&D Laboratory, Brockton / West Roxbury VA Medical Center, West Roxbury, and Department of Orthopedic Surgery, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115; and ‡Pain Research Center, Department of Anesthesiology, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115*

Received March 27, 1998; accepted September 4, 1998

Kinetics of myelinated axon synthesis

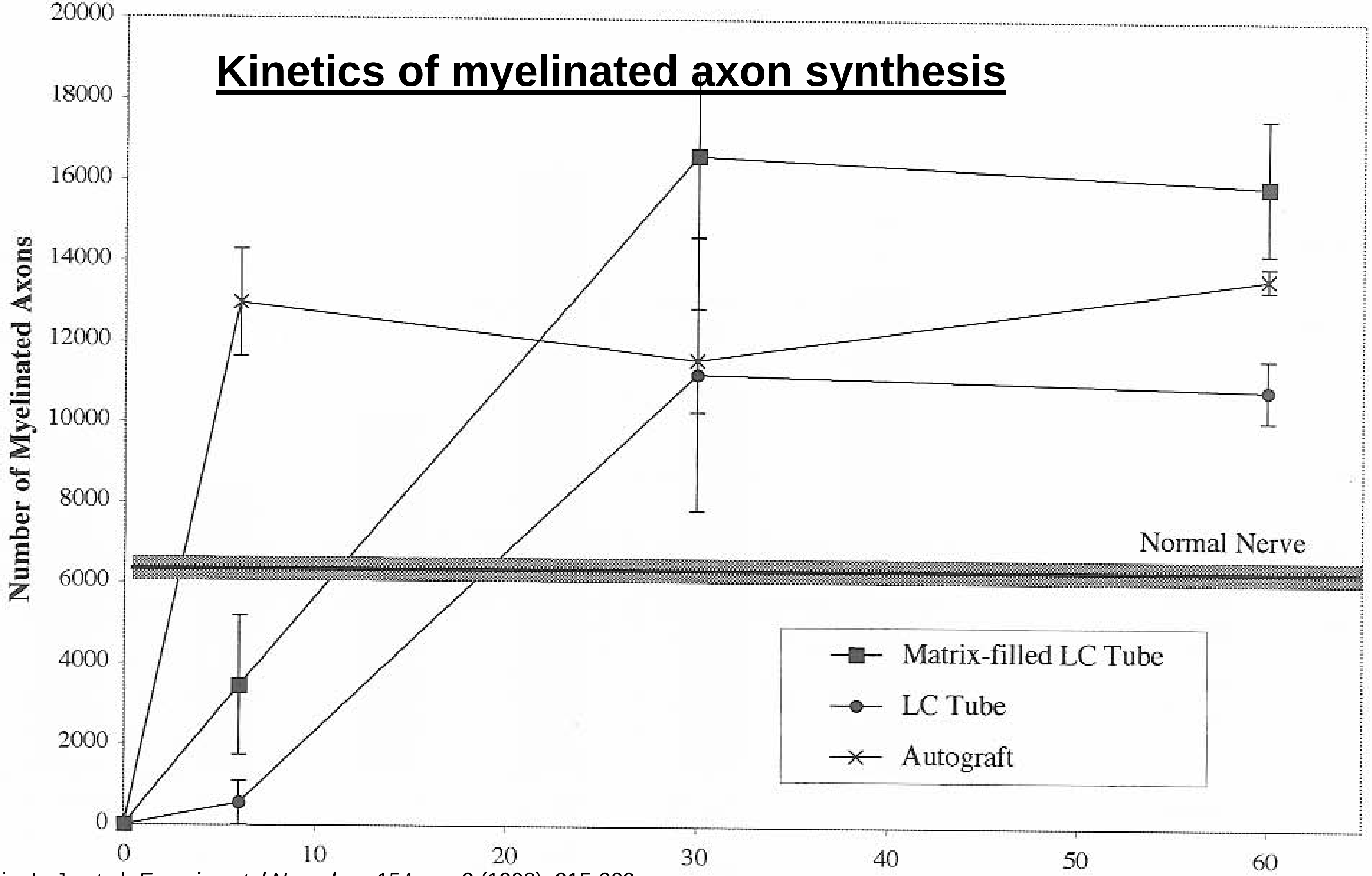


FIG. 2. Total number of myelinated axons as a function of time for the LC/M, LC, and AG groups. For each group, both a growth region and a plateau region were observed. In the tubulated groups, the number of axons per nerve increased up to 30 weeks and remained unchanged thereafter ($P > 0.3$). In contrast, values at the autografted sites reached apparently constant values after only 6 weeks ($P > 0.4$). The 6-week data, described in detail previously (15), have been presented here for reference only. LC, large-pore collagen.

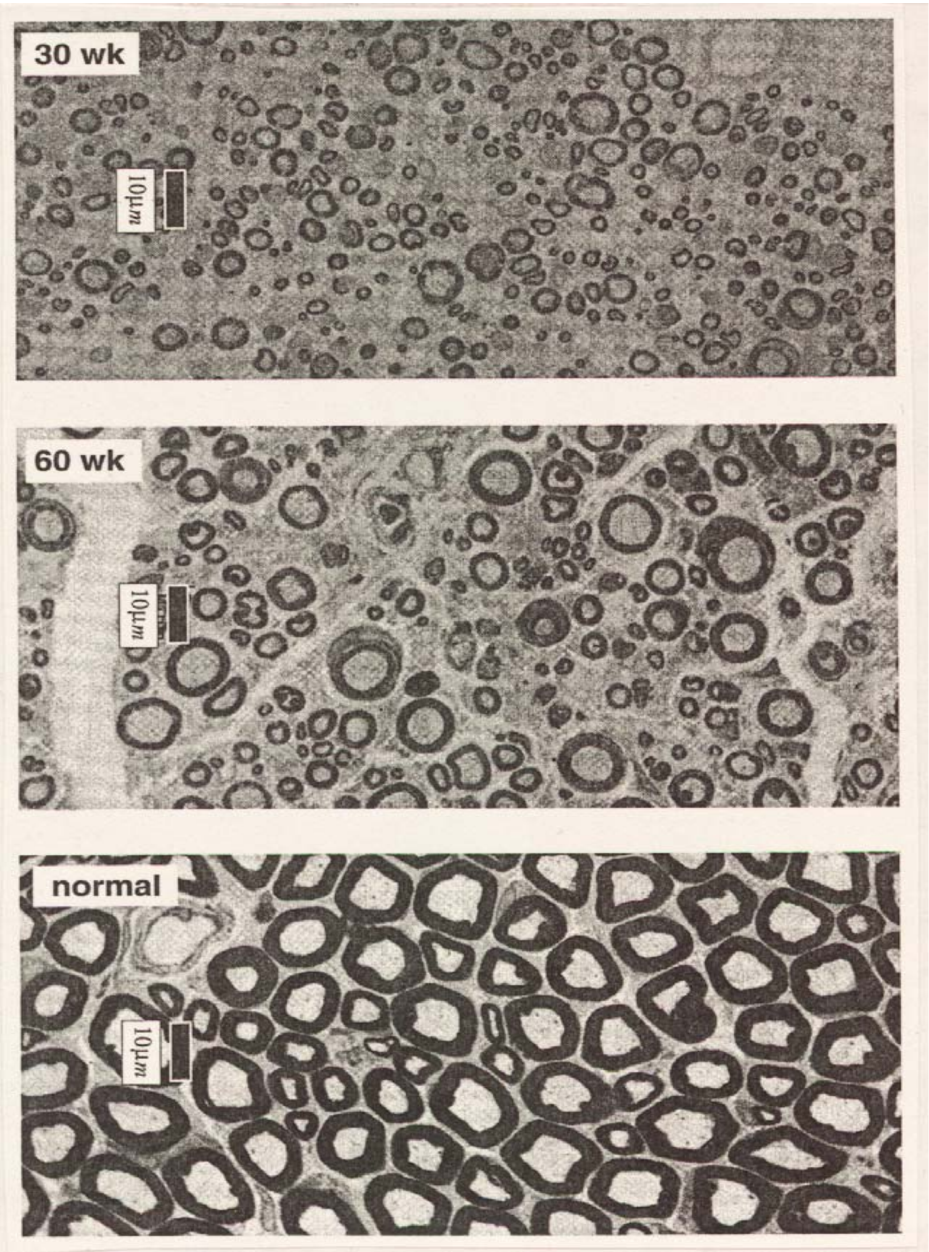
Effect of device composition on number of myelinated axons (60 weeks)

Axon diameter distribution for various devices (60 weeks)

Kinetics of synthesis of large diameter (≥6 μm) nerve fibers

Effect of various devices on number of large diameter fibers (60 weeks)

KINETICS OF NERVE SYNTHESIS



30 weeks

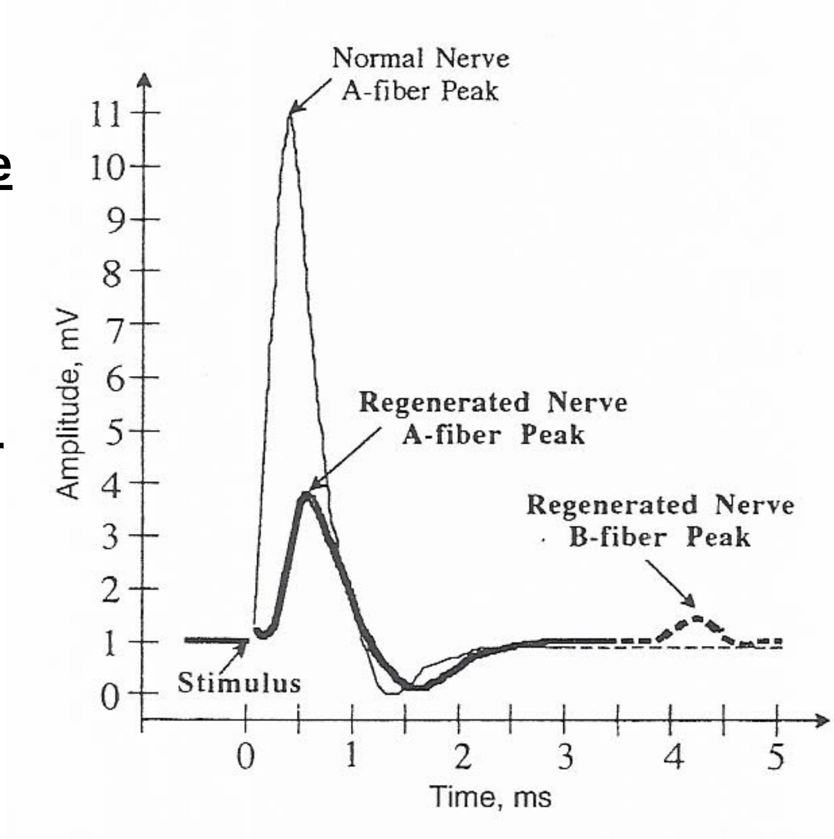
60 weeks

Normal

Chamberlain, L. J., et al. *Experimental Neurology* 154, no. 2 (1998): 315-329.

<u>Electrophysiological behavior of normal (light line) and regenerated nerve (dark line)</u>

Regenerated nerve is weaker (lower peak amplitude) and slower (delayed peaking)



Y-axis: Amplitude (strength) of transmitted electric signal
X-axis: Time following stimulation (at 0 ms)

Chamberlain, L. J., et al. *Experimental Neurology* 154, no. 2 (1998): 315-329.

6. Comparative regenerative activity of various devices
(Table 6.1, pp. 147-8)

What does each of these device features contribute to the quality of regeneration? Compare values of L_c and ΔL.

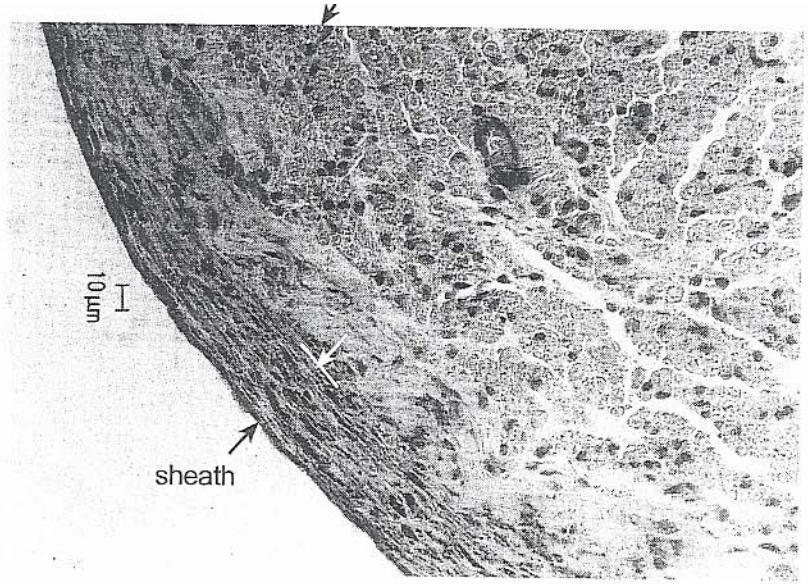
- **Tubulation**
- **Tube wall composition**
- **Tube wall permeability**
- **Fillings: Schwann cells, solutions of proteins, gels based on ECM components, insoluble substrates**

Tube wall composition and permeability

- Bridging the two stumps with a tube, almost any kind of tube, greatly improves quality of regeneration.
- Tube wall composition is critically important. Silicone tubes without holes are greatly inferior to collagen tubes fabricated from porous scaffolds.
- Increase of cell (but not protein) permeability of silicone tubes improved quality.

Silicone tube

Partly regenerated rat sciatic nerve. Tubulated in silicone tube.



cross-section shows thick sheath of contractile cells

Chamberlain, L. J., et al. *J Comp Neurol* 417, no. 4 (2000): 417-430.

See also Fig. 4.5 and discussion in [TORA]

Silicone tube

Contractile cells (brown) ensheathe regenerating stump of transected rat sciatic nerve

Image removed due to copyright restrictions. See Fig. 4.6 in [TORA].

near original proximal stump

near original distal stump

Tube fillings

- Schwann cells, growth factors (aFGF and bFGF) and several insoluble substrates increased quality of regeneration, sometimes greatly.
- NGF had no effect.
- Gels based on ECM components (collagen, fibronectin, laminin) had no effective or impeded regeneration.

Regeneration across a 15 mm gap (very long) bridged by a silicone tube

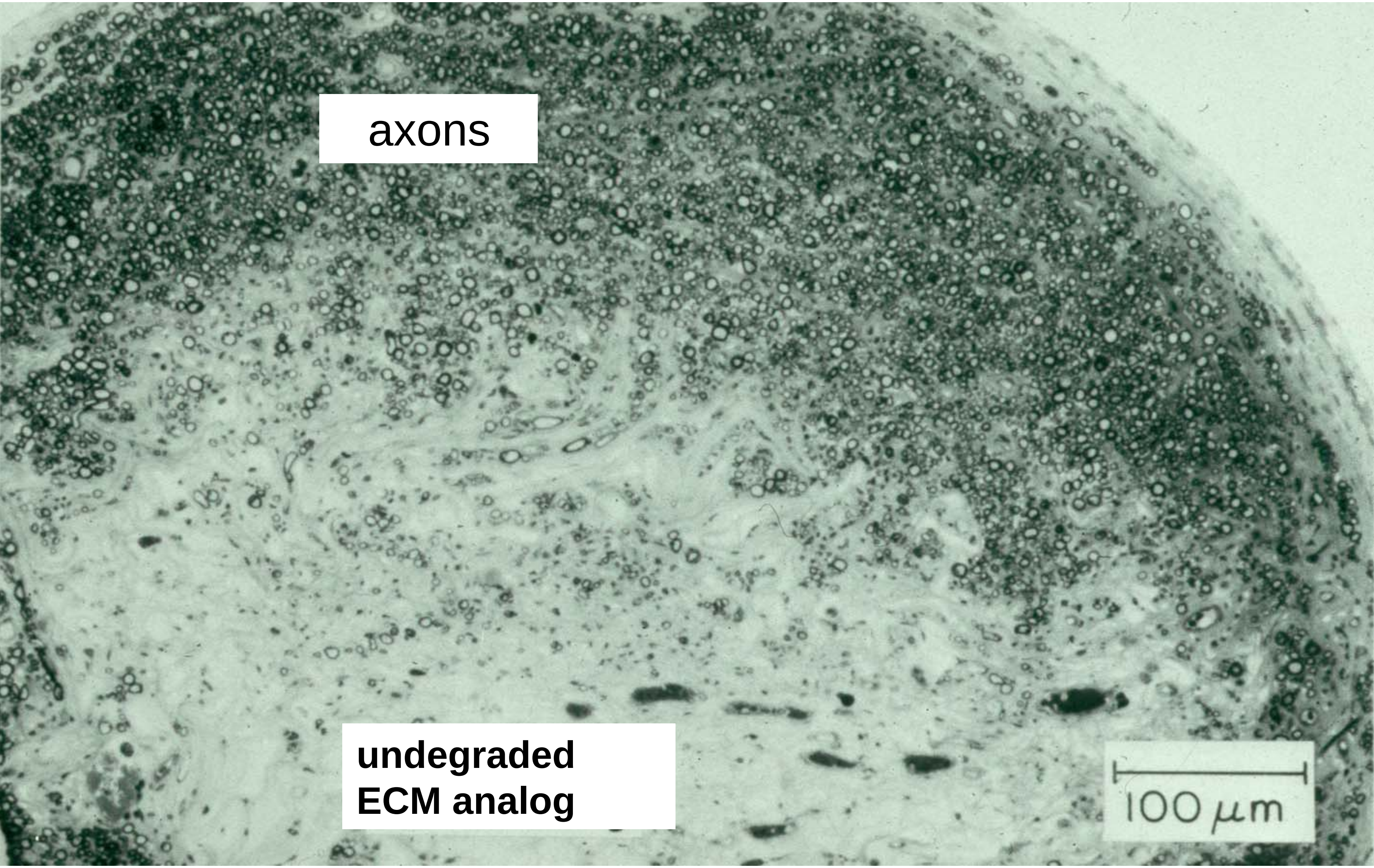
filled
with
scaffold

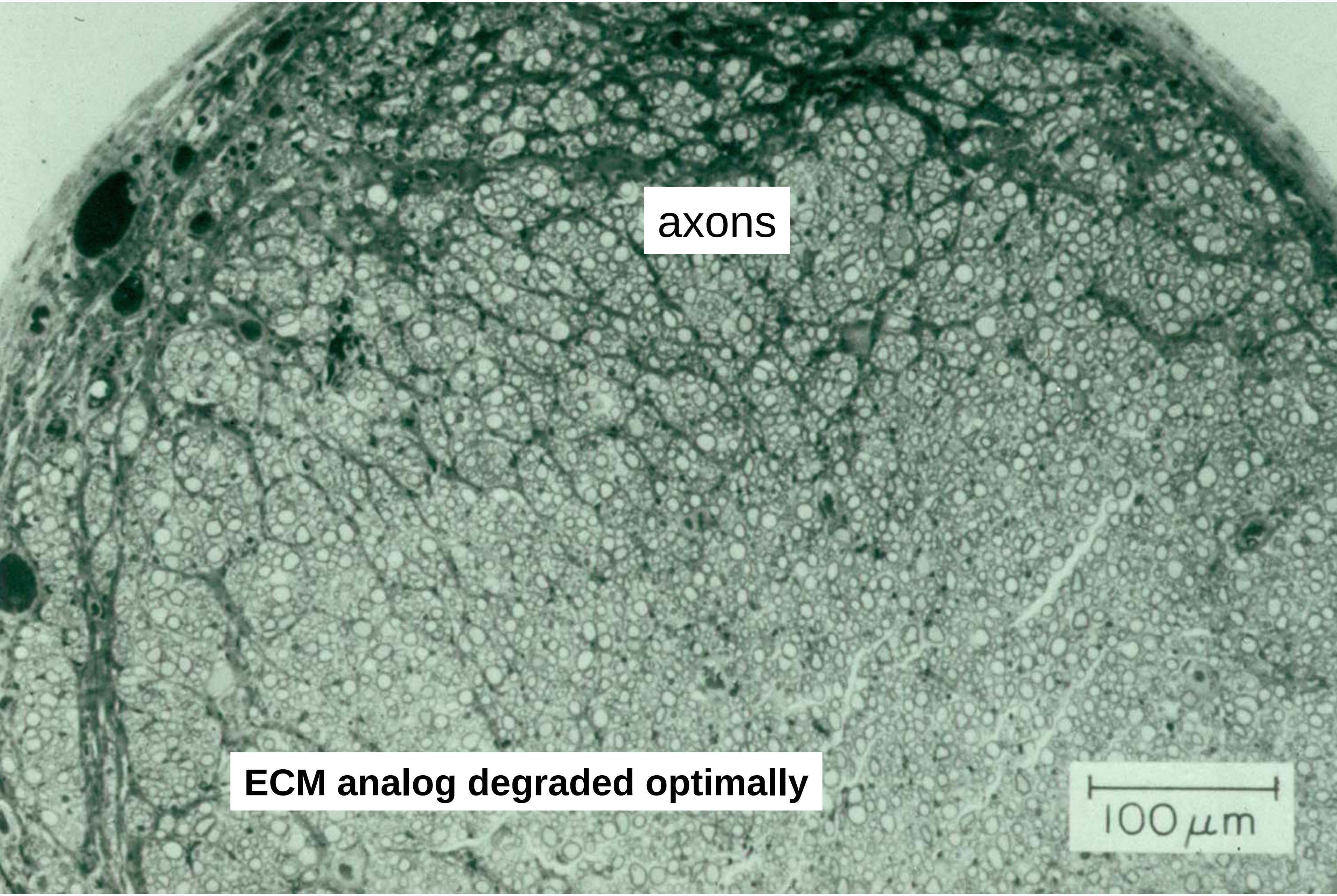
Photo removed due to copyright restrictions.

unfilled

Effect of degradation rate of tube filling based on a porous ECM analog (NRT)

- **Undegraded ECM analog physically impeded axon elongation.**
- **Optimal quality of regeneration obtained with ECM analog that degraded at an <u>intermediate</u> rate.**

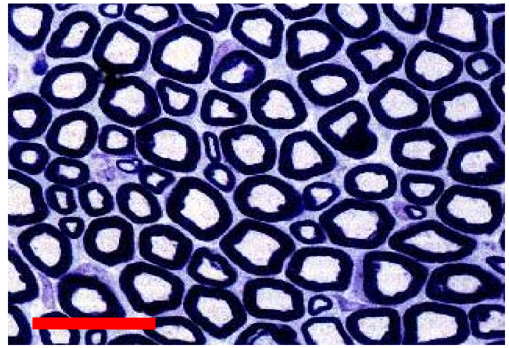

axons
undegraded
ECM analog
100 μm


axons
ECM analog degraded optimally
100 µm

Histomorphometry

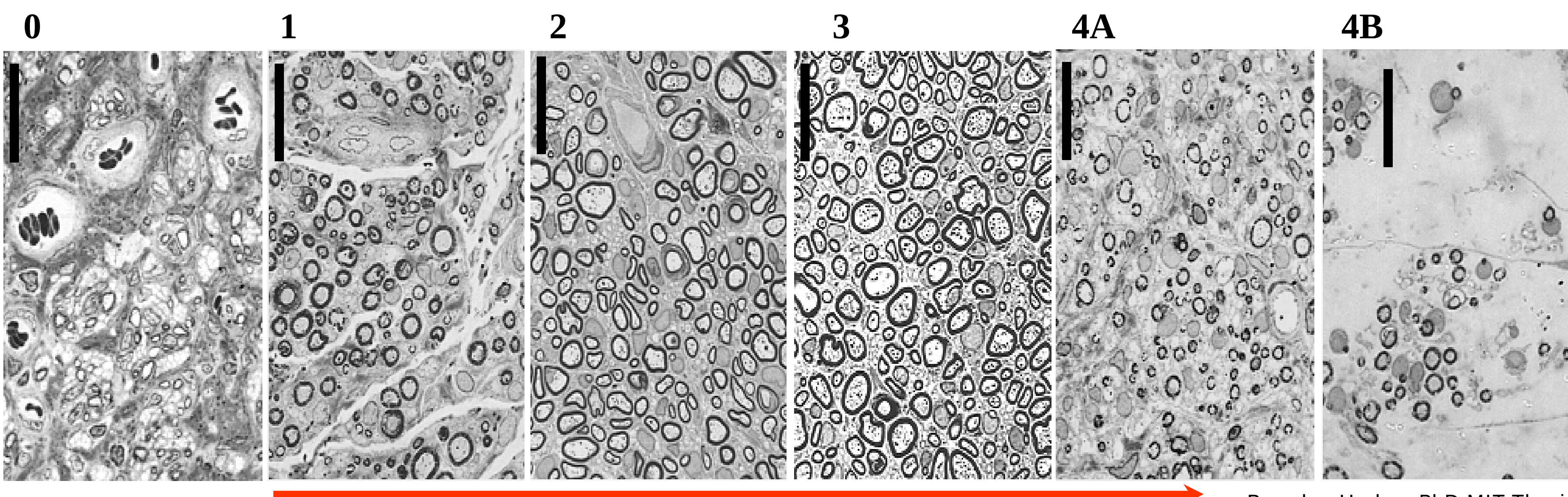
Normal Sciatic Nerve
(Chamberlain, 2000)

Scale bars: 25 µm



Chamberlain, L. J., et al. *Experimental Neurology* 154, no. 2 (1998): 315-329.
Courtesy of Elsevier, Inc., http://www.sciencedirect.com. Used with permission.

#3 is best!



Brendan Harley, PhD MIT Thesis.

Decreasing tube degradation rate

Effect of pore diameter and degradation rate on inverse conduction velocity (latency)

Graph removed due to copyright restrictions.
See Figure 10.9 in [TORA].

Structural features of ECM analogs used as tube fillings in <u>nerve</u> regeneration

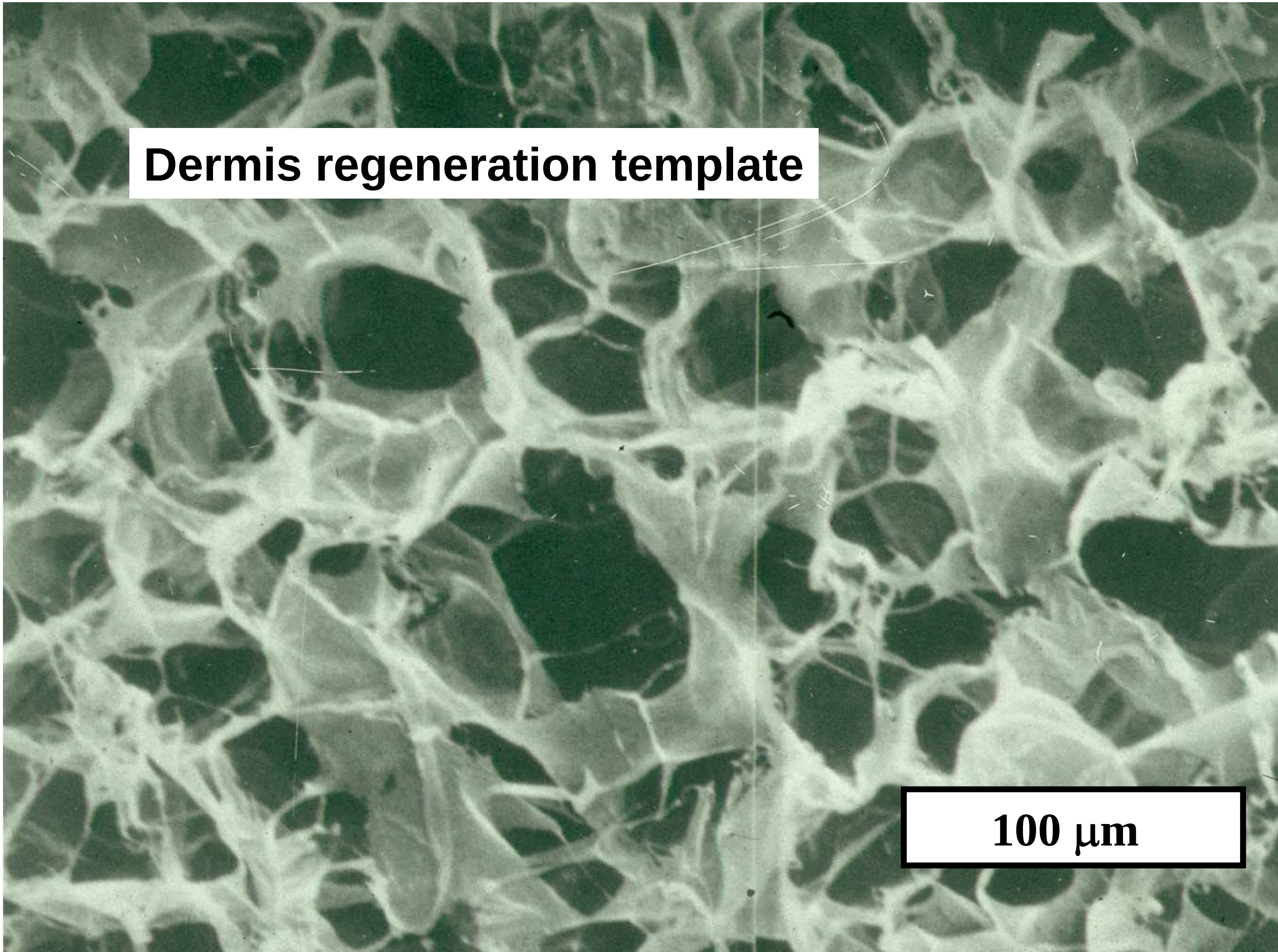
1. pore structure (ligand density)

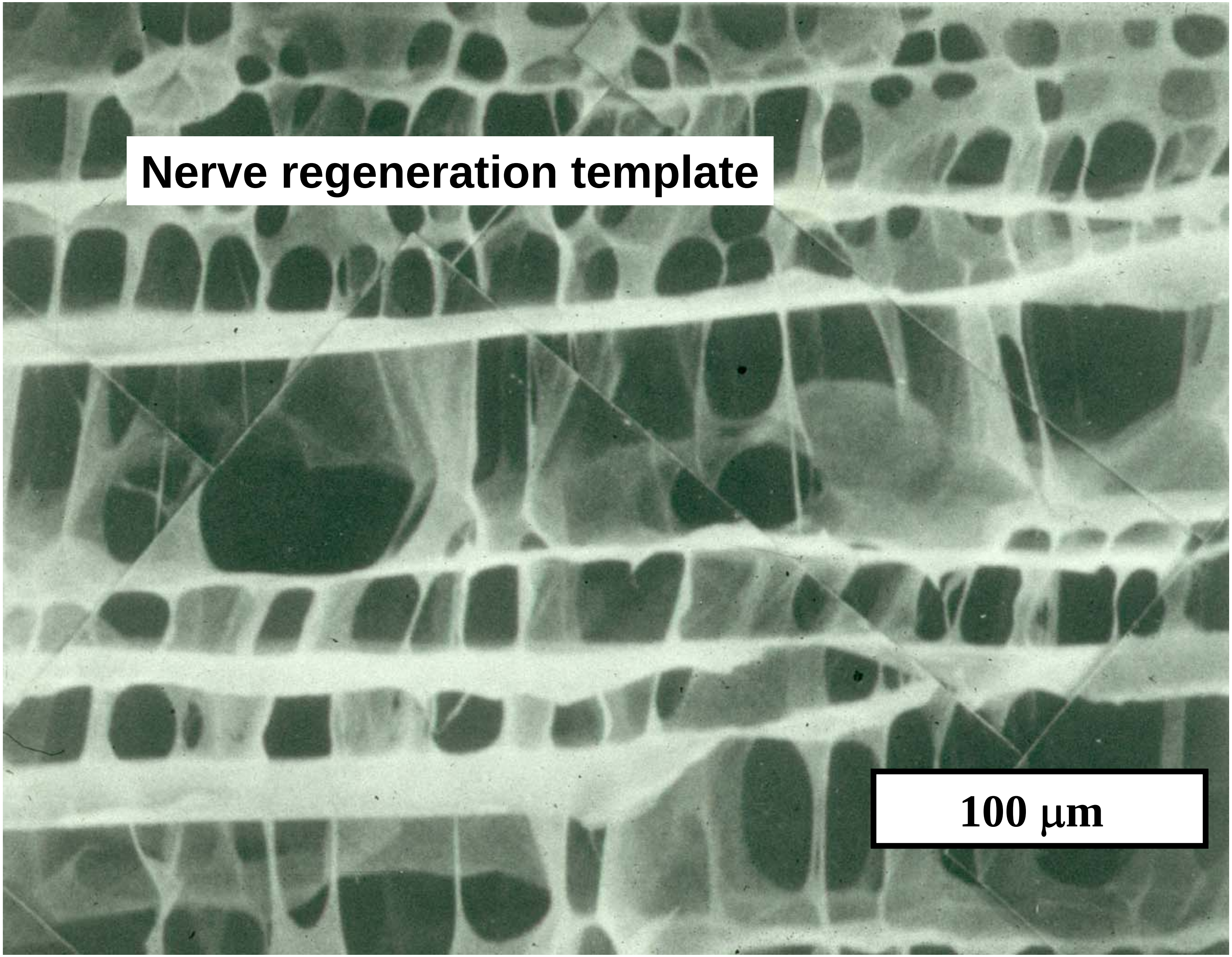
Diagram removed due to copyright restrictions.

2. macromolecular structure (ligand duration)

3. chemical composition (ligand identity)

4. orientation of pore channel axes


Dermis regeneration template
100 μm


Nerve regeneration template
100 μm

Summary of results*

- **Tube presence was essential**
- **Tube wall composition: collagen > degradable synthetic polymer > silicone.**
- **Tube wall permeability: cell-permeable > protein permeable > impermeable.**
- **Tube fillings:**

  --- suspensions of Schwann cells

  --- solution of either aFGF or bFGF (not NGF!)

  --- crosslinked ECM networks > ECM gels

  --- thin polymeric filaments oriented along tube axis

  --- highly porous, insoluble ECM analogs with appropriately small pore diameter, axial orientation of pore channel axes and critically adjusted degradation rate.

---

*Yannas, Zhang and Spilker, 2007. "Standardized criterion to analyze and directly compare various materials and models for peripheral nerve regeneration." *Journal of Biomaterials Science, Polymer Edition* 18, no. 8 (2007): 943-966.

20.441J / 2.79J / 3.96J / HST.522J Biomaterials-Tissue Interactions
Fall 2009