Simplest synthetic pathways*---outline

A. Symbolism of organ synthesis.

B. The central question of organ synthesis.

C. What is required to synthesize an organ?

D. Trans-organ rules of synthesis.

*_Tissue and Organ Regeneration in Adults_, Yannas IV, New York, Springer, 2001

A. Symbolism of Organ Synthesis

Information stored in a chemical equation

Ammonia synthesis (F. Haber)

$$3H_2 + N_2 \xrightarrow{T,\ P} 2NH_3$$

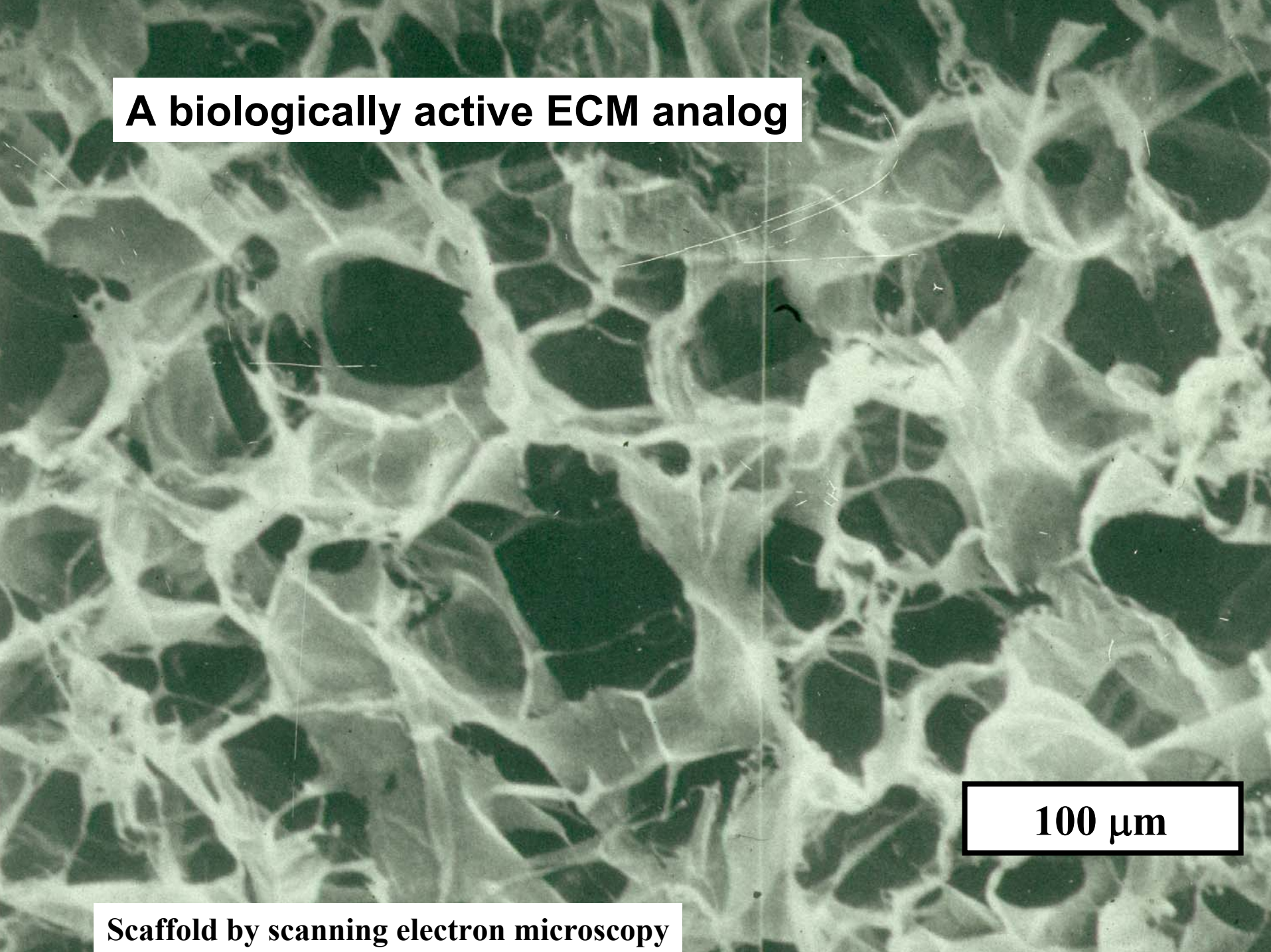
reactor

reactants → products

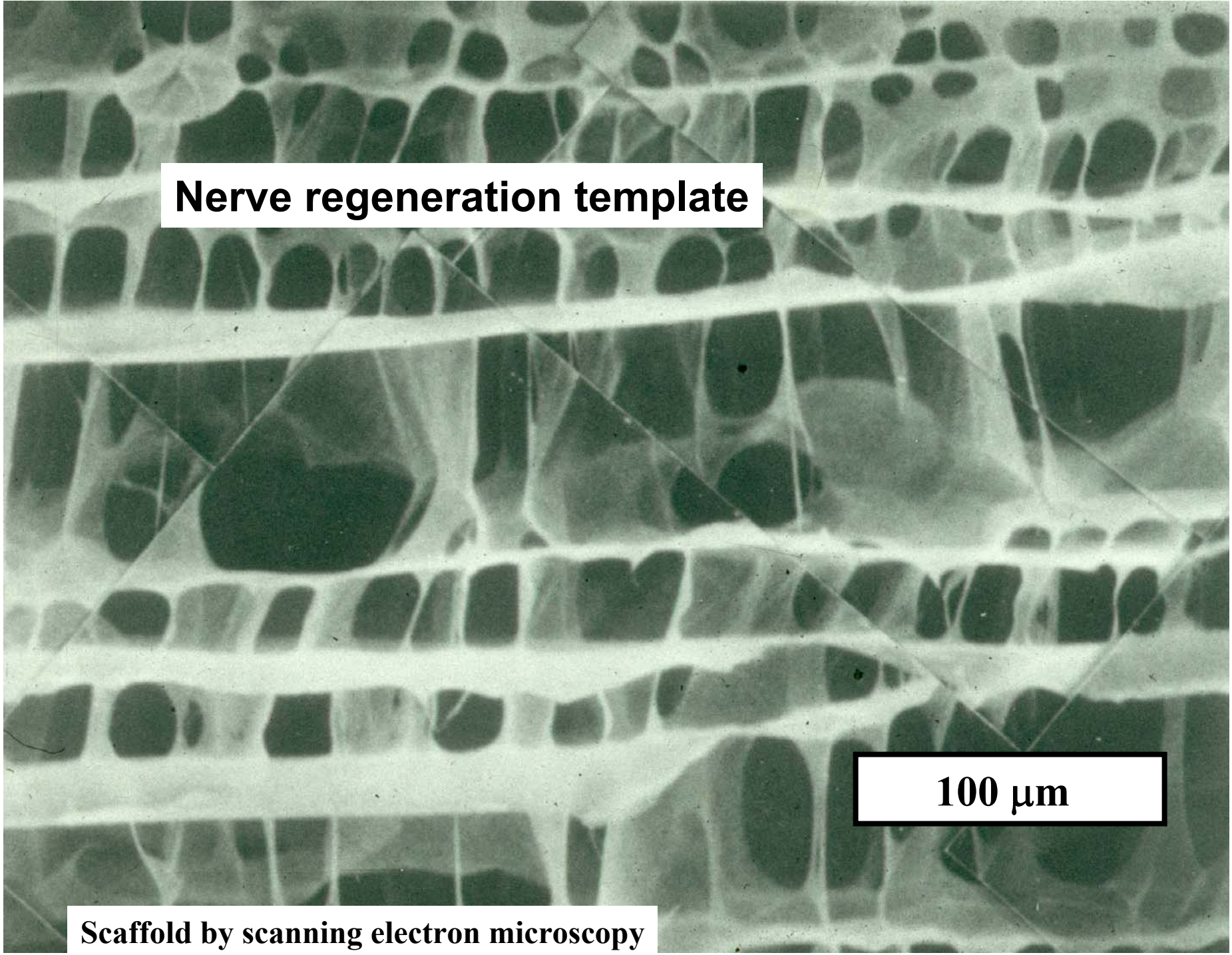
NOTE: The stoichiometry (masses on both sides) of a chemical equation expresses conservation of mass (Lavoisier)

Transition to biology
I. Reactants

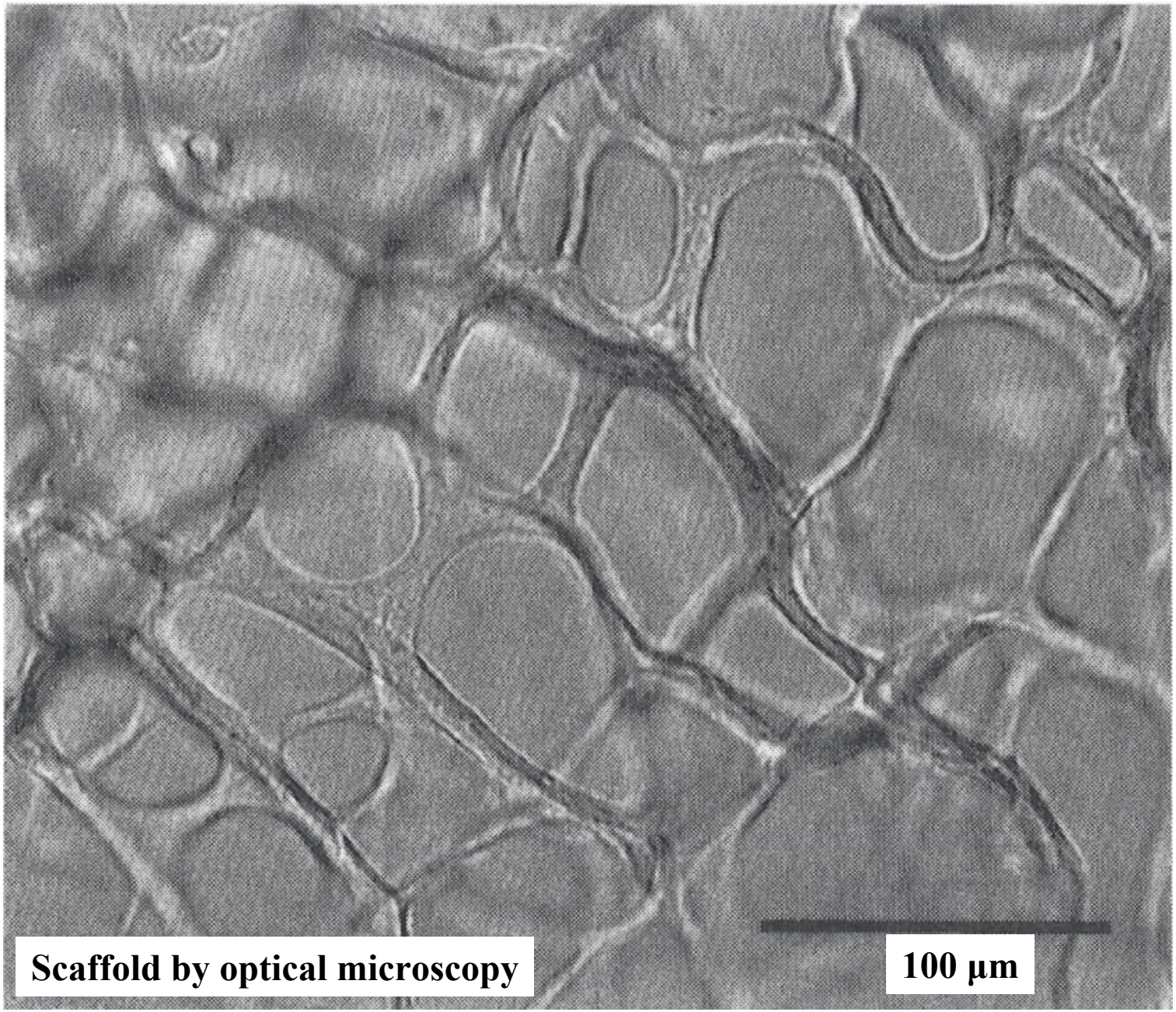
- **Cells migrate, proliferate, synthesize matrices and cytokines, degrade matrices, etc.**
- **Cytokines are soluble molecules that diffuse. They serve as "language" between cells.**
- **Matrices are insoluble macromolecular networks and do not diffuse. They control cell behavior (phenotype) via integrin-ligand binding. Usually porous ("scaffolds").**



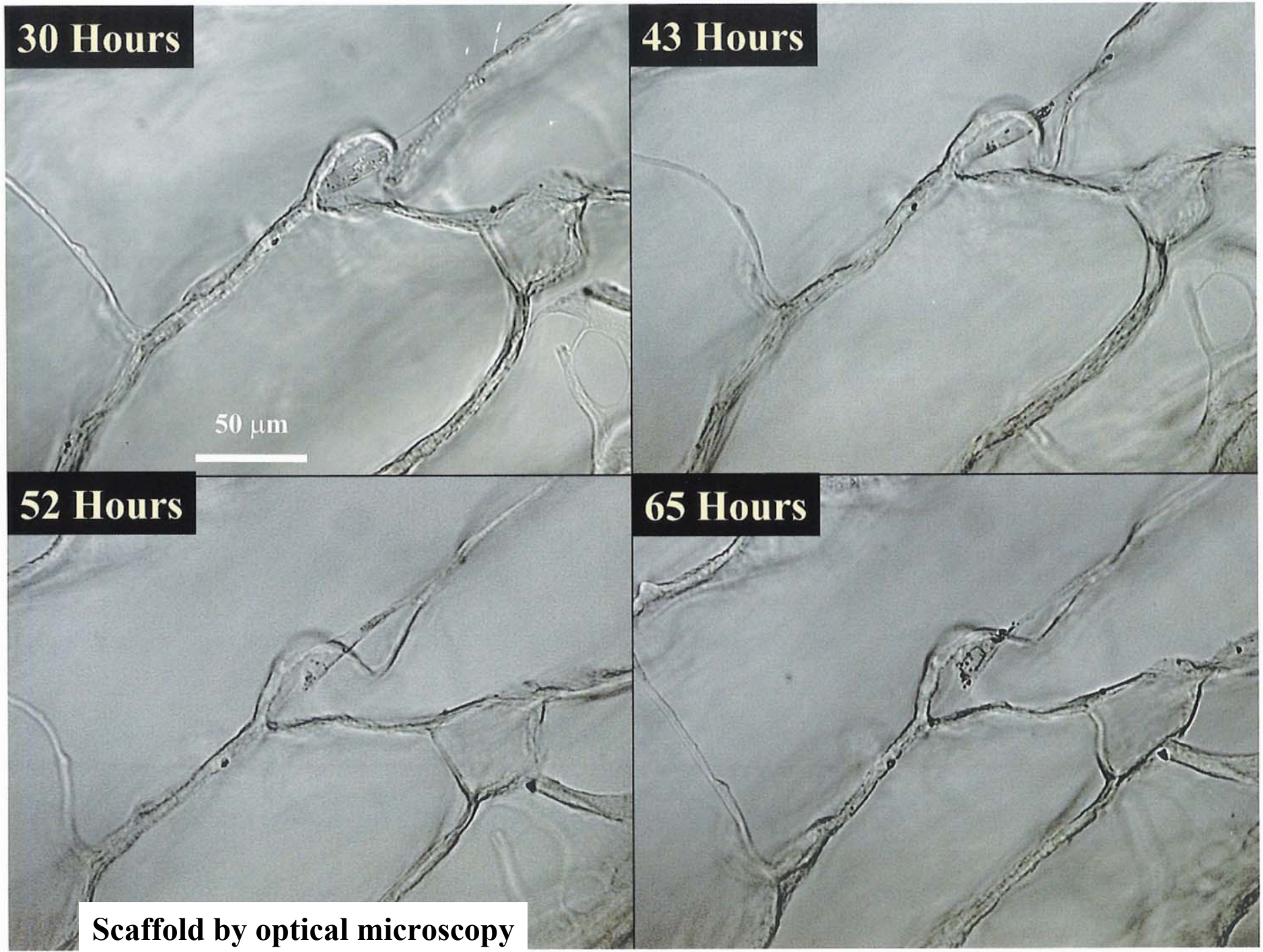
Scaffold by scanning electron microscopy



Scaffold by scanning electron microscopy

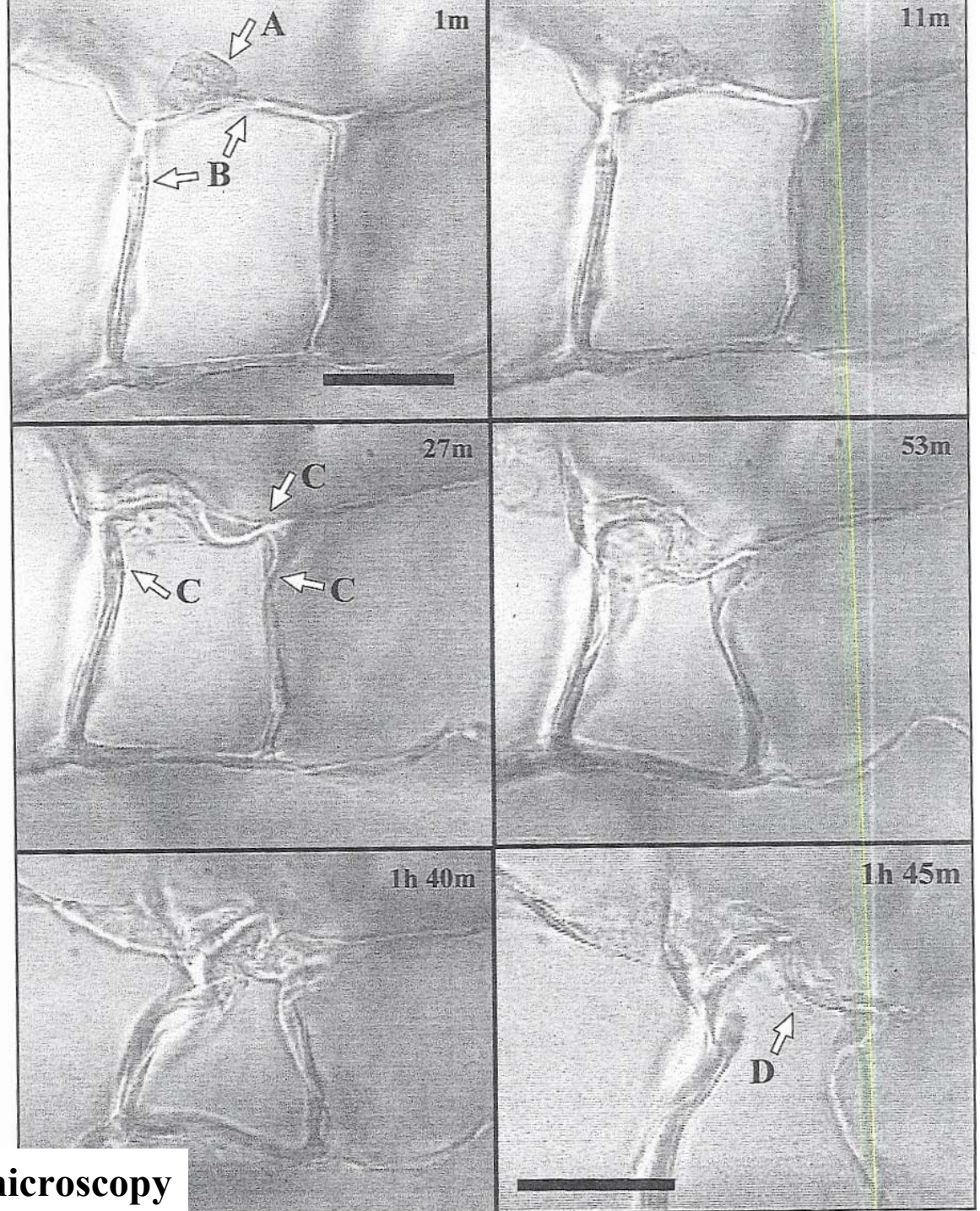


Source: Freyman, T. M., I. V. Yannas, R. Yokoo, and L. J. Gibson. "Fibroblast contraction of a collagen-GAG matrix." *Biomaterials* 22 (2001): 2883-2891. Courtesy Elsevier, Inc., http://www.sciencedirect.com. Used with permission.



Scaffold by optical microscopy

Another sequence showing a cell (A) elongating and deforming matrix struts (B)



Scaffold by optical microscopy

Transition to biology
II. Reactors

- In vitro reactors are dishes or flasks for cell culture.
- In vivo reactors are anatomical sites of organ loss in the living organism.
- Experimental in vivo reactors are generated by surgical excision (scalpel, laser, etc.).
- When organ synthesis takes place in vivo at the correct anatomical site of living organism it is referred to as “induced regeneration”.

Skin: In vitro or in vivo synthesis?

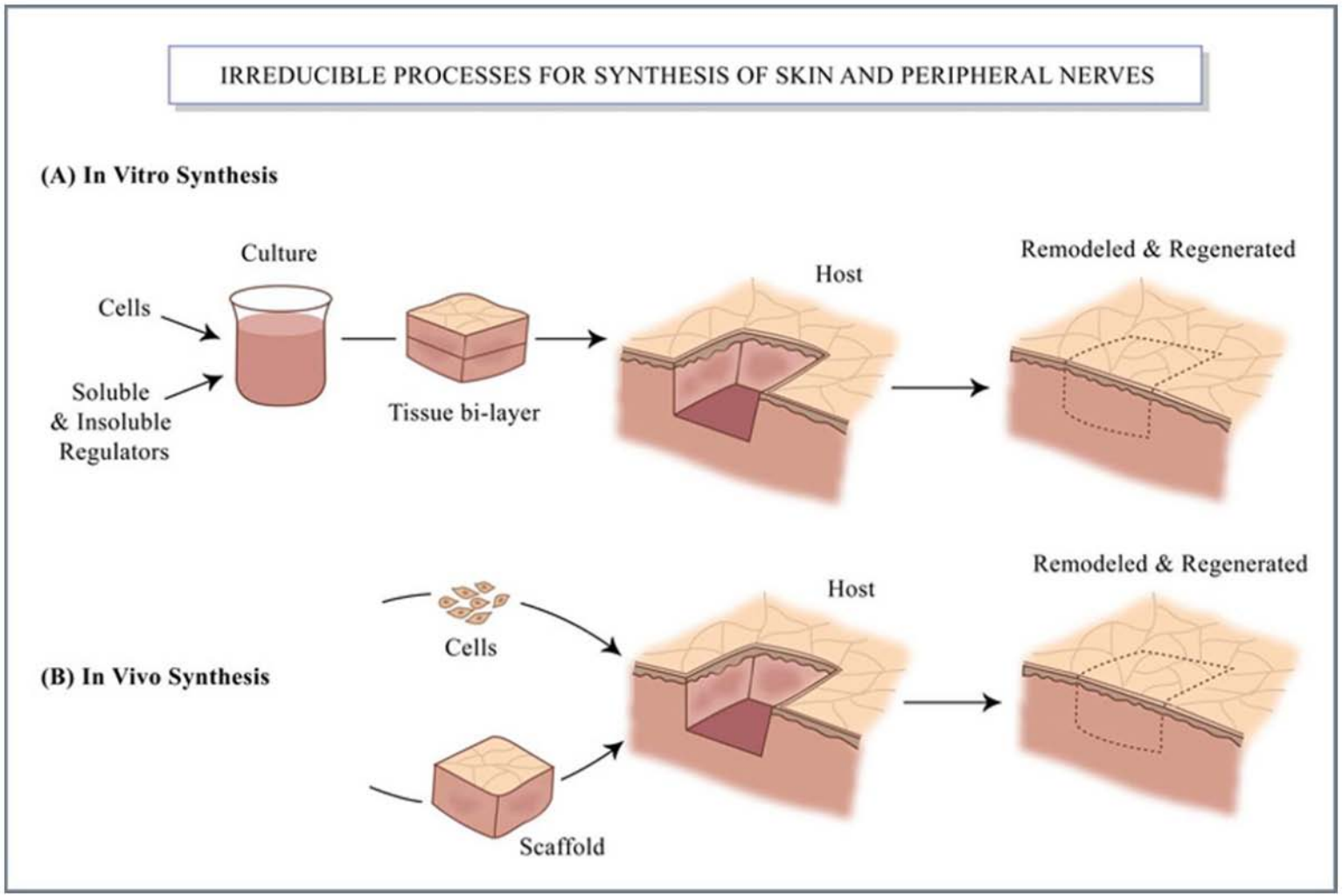


Figure by MIT OpenCourseWare.

Nerves: In vitro or in vivo?

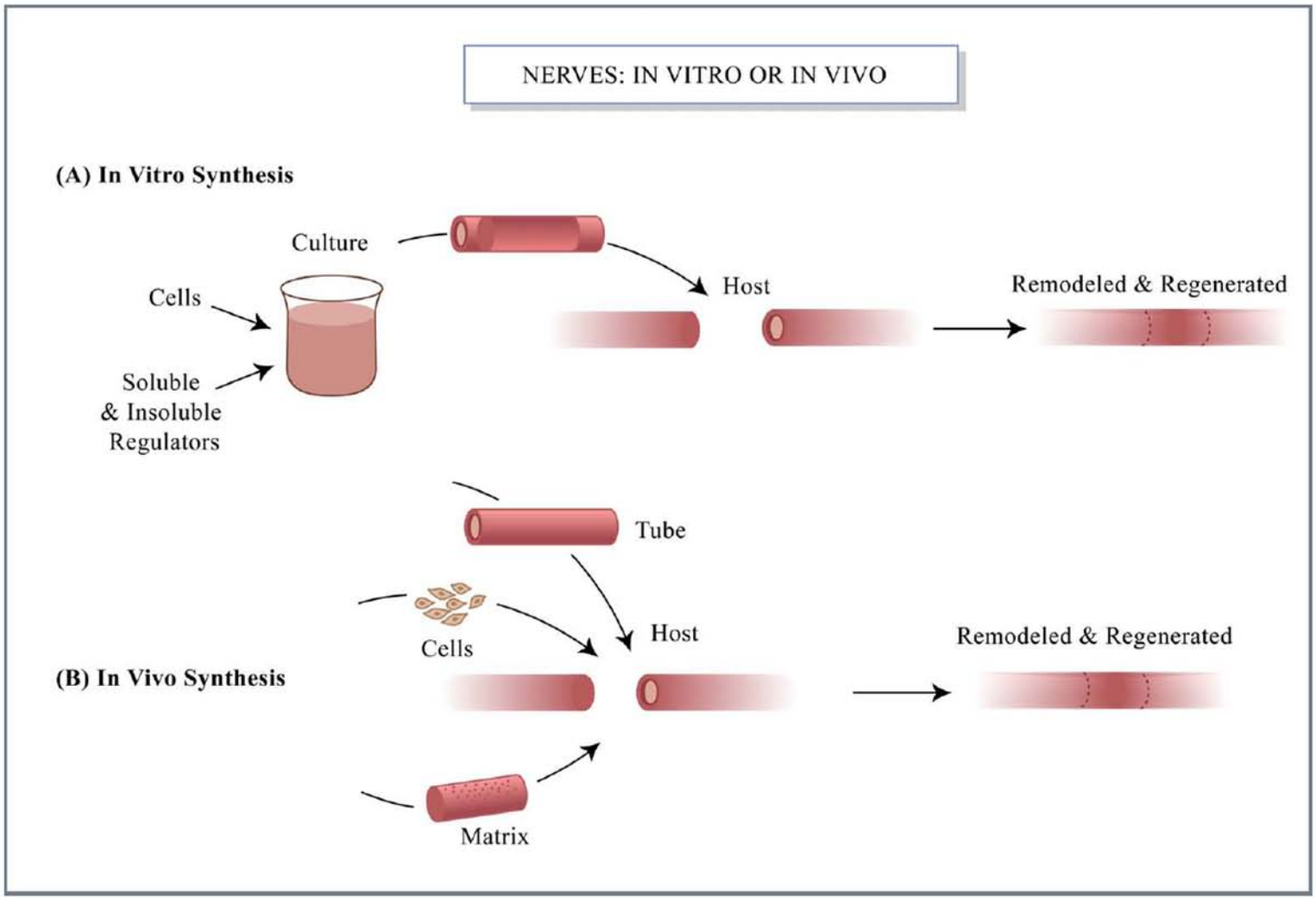
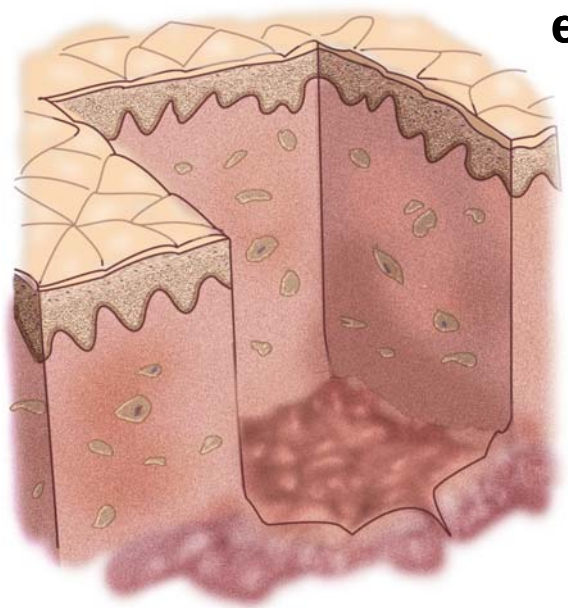


Figure by MIT OpenCourseWare.

Standardized in vivo reactors for study fo skin synthesis and peripheral nerve synthesis

SKIN



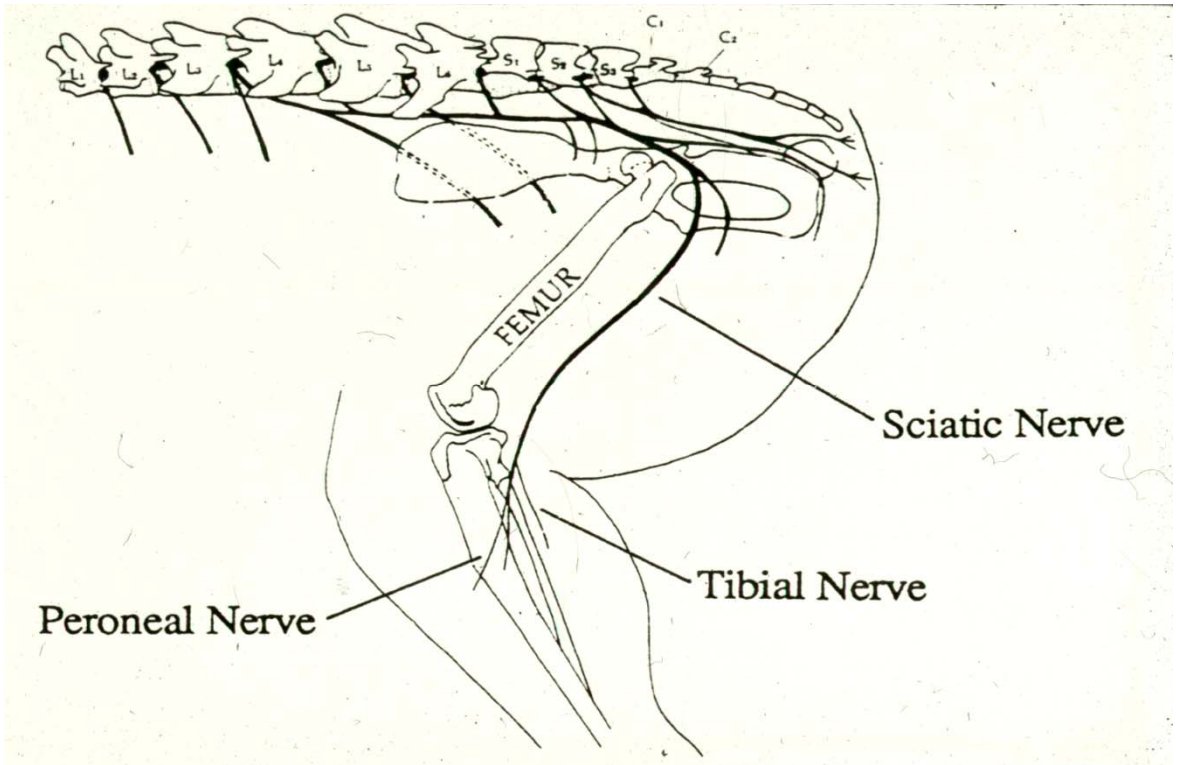
epidermis

dermis

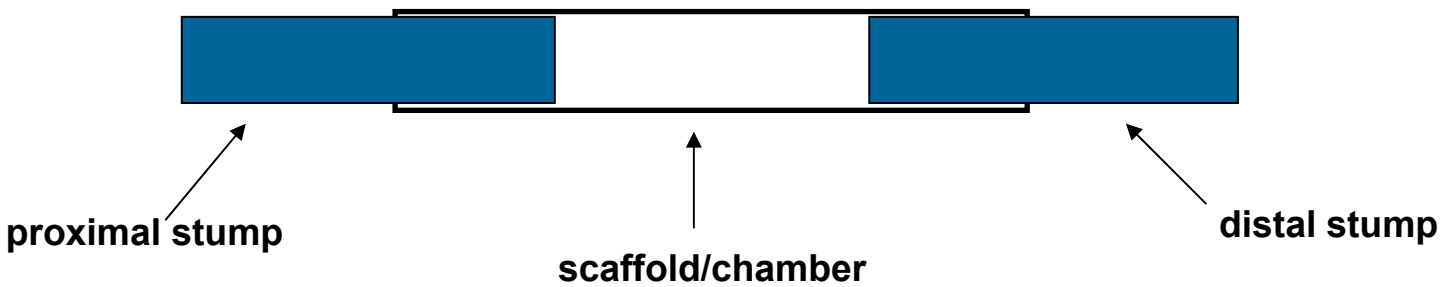
PERIPHERAL NERVE

nerve stumps

Rat sciatic nerve model



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



Transition to biology.
III. Products

- Organs are made up of tissues.
- Products of the synthesis can be tissues or organs.
- Almost all organs are essentially made up of three types of tissues: epithelial, basement membrane and stroma (connective tissue).
- Describe degree of completion of product of synthesis using the triad.

Three histology images removed due to copyright restrictions.
See Butler, CE, et al. “Effect of Keratinocyte Seeding of Collagen-Glycosaminoglycan Membranes on the Regeneration of Skin in a Porcine Model.” *Plast. Reconstr. Surg*. 101, no. 6 (May 1998): 1572-1579.

Scaffold seeded with epithelial cells

KINETICS OF SKIN SYNTHESIS I.

Epithelial tissue being synthesized together with stroma

Scaffold slowly degrading

Butler et al., 1998

Three histology images removed due to copyright restrictions.
See Butler, CE, et al. “Effect of Keratinocyte Seeding of Collagen-Glycosaminoglycan Membranes on the Regeneration of Skin in a Porcine Model.” *Plast. Reconstr. Surg*. 101, no. 6 (May 1998): 1572-1579.

KINETICS OF SKIN SYNTHESIS II.

Epithelial tissue separating out from stroma

Scaffold degraded; diffuses away

Butler et al., 1998

Partially regenerated skin is not scar.
Scar does not have capillary loops. Nor does scar have a wavelike border separating epidermis from dermis

Diagram removed due to copyright restrictions. See Figure 5.2a in [TORA].

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

capillary loops

Normal skin has capillary loops and a wavelike border separating epidermis from dermis. Burkitt et al., 1992

Histology photo removed due to copyright restrictions. See Compton, C.C., et al. *J. Invest. Dermatol*. 110 (1998): 908-916.

capillary loops

75 μm

Partially regenerated skin in the swine. Compton et al., 1998

Partially regenerated skin is not scar.

Study blood vessels at interface of epidermis-dermis.
Scar has no blood vessels at interface.
Regenerated skin is not scar.

normal skin (guinea pig)

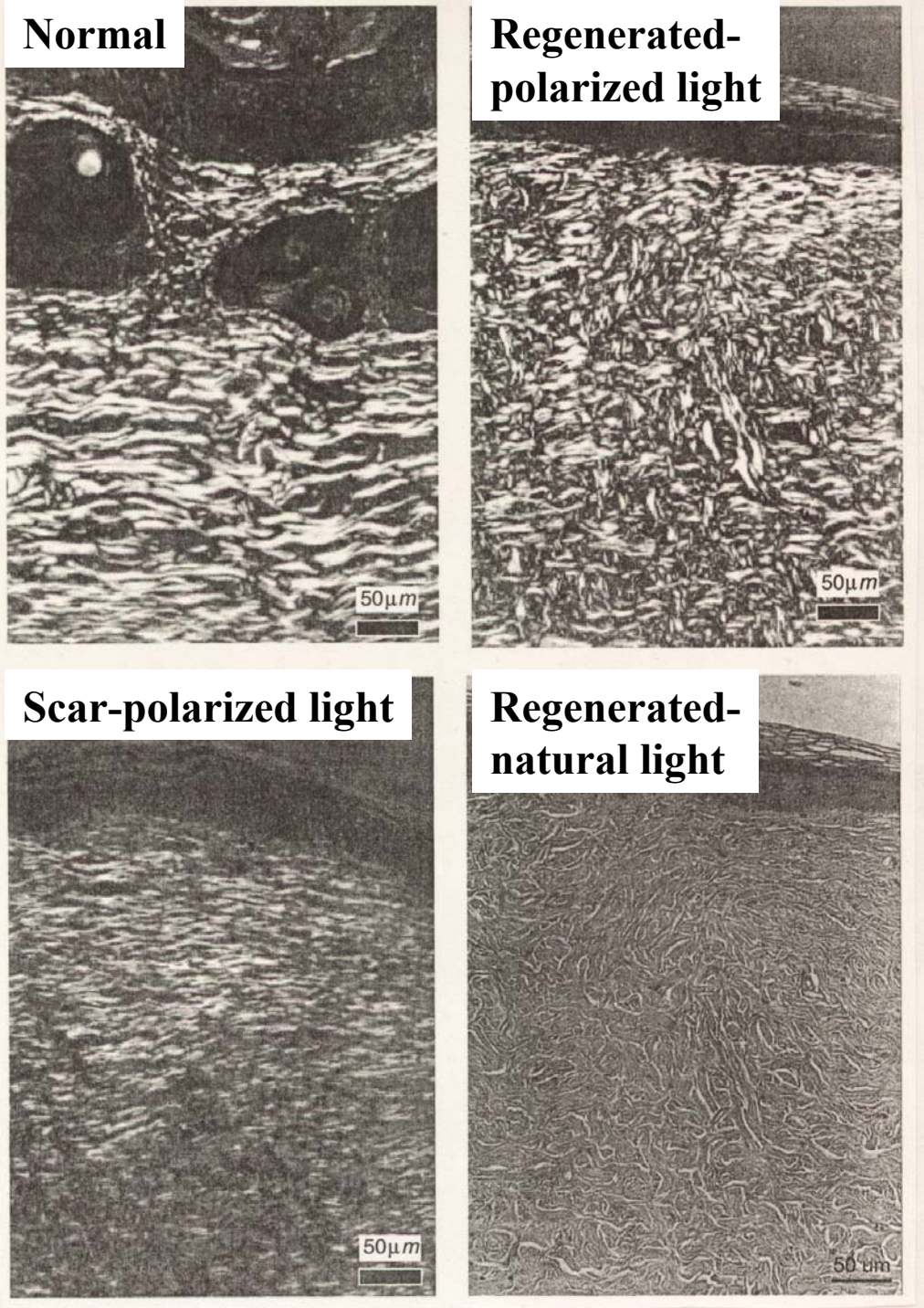
Three histology photos removed due to copyright restrictions. See Figure 5.4 in [TORA].

scar

v, blood vessels (absent in scar)
d, dermis

regenerated skin

Comparison of stroma (dermis) in regenerated skin, normal skin and scar (guinea pig)



Orgill, D. P. MIT
PhD Thesis, 1983.

Normal Dermis

Scar

Diagram removed due to copyright restrictions.
Schematic of laser beam passing through histologic slide.
See Fig. 4.7 in [TORA].

Images removed due to copyright restrictions.
Laser scattering patterns
See Fig. 4.7 in [TORA].

$$S = 2\left\langle \cos^2(a) \right\rangle - 1$$

	Dermis	Scar
$\langle \cos^2(a) \rangle$	0.5	1
Orientation function, S	0	1

Identify scar using laser light scattering assay

Ferdman and Yannas, 1993

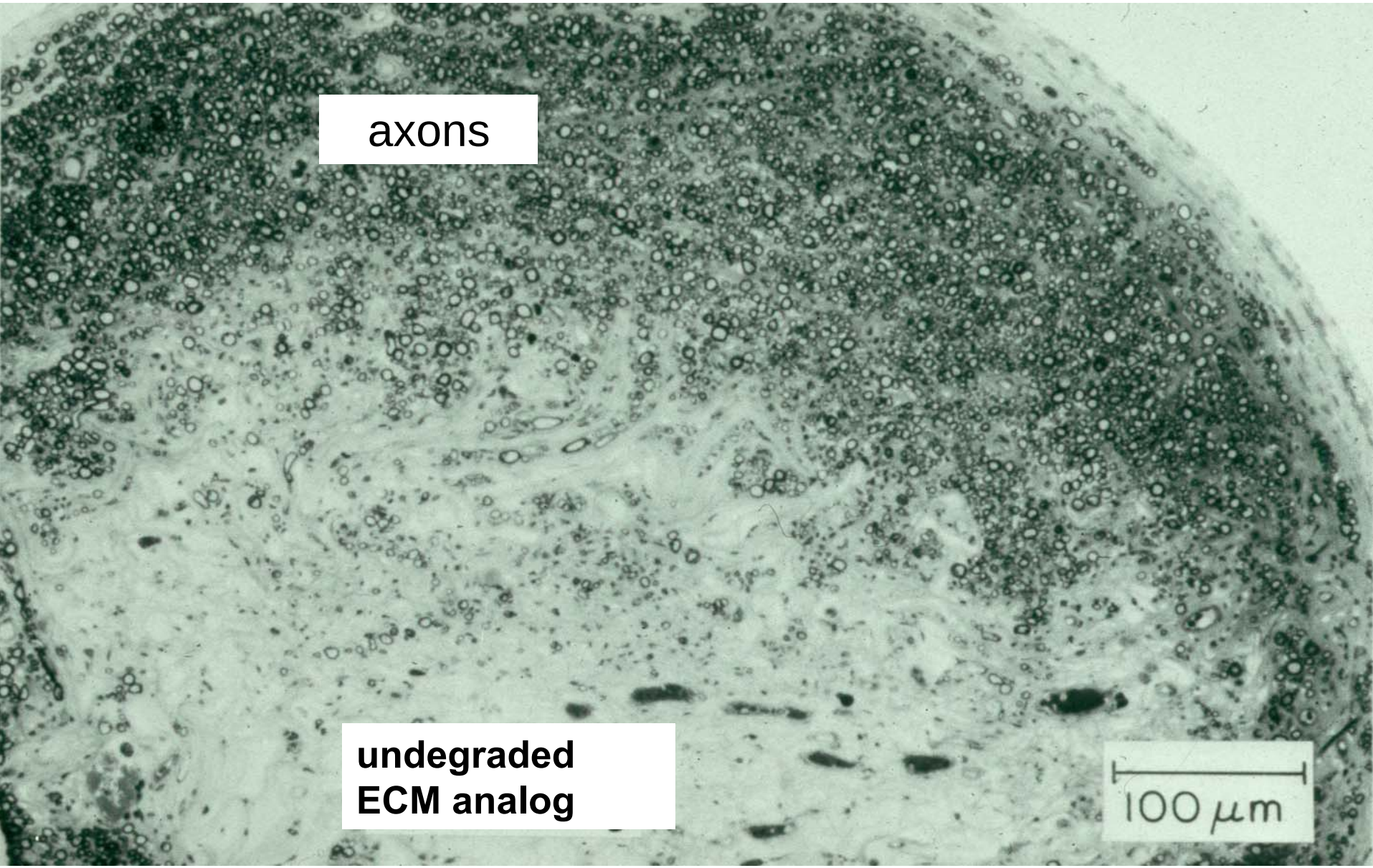
Gross view of regeneration across a 10 mm gap bridged by a silicone tube

Silicone tube
filled with scaffold

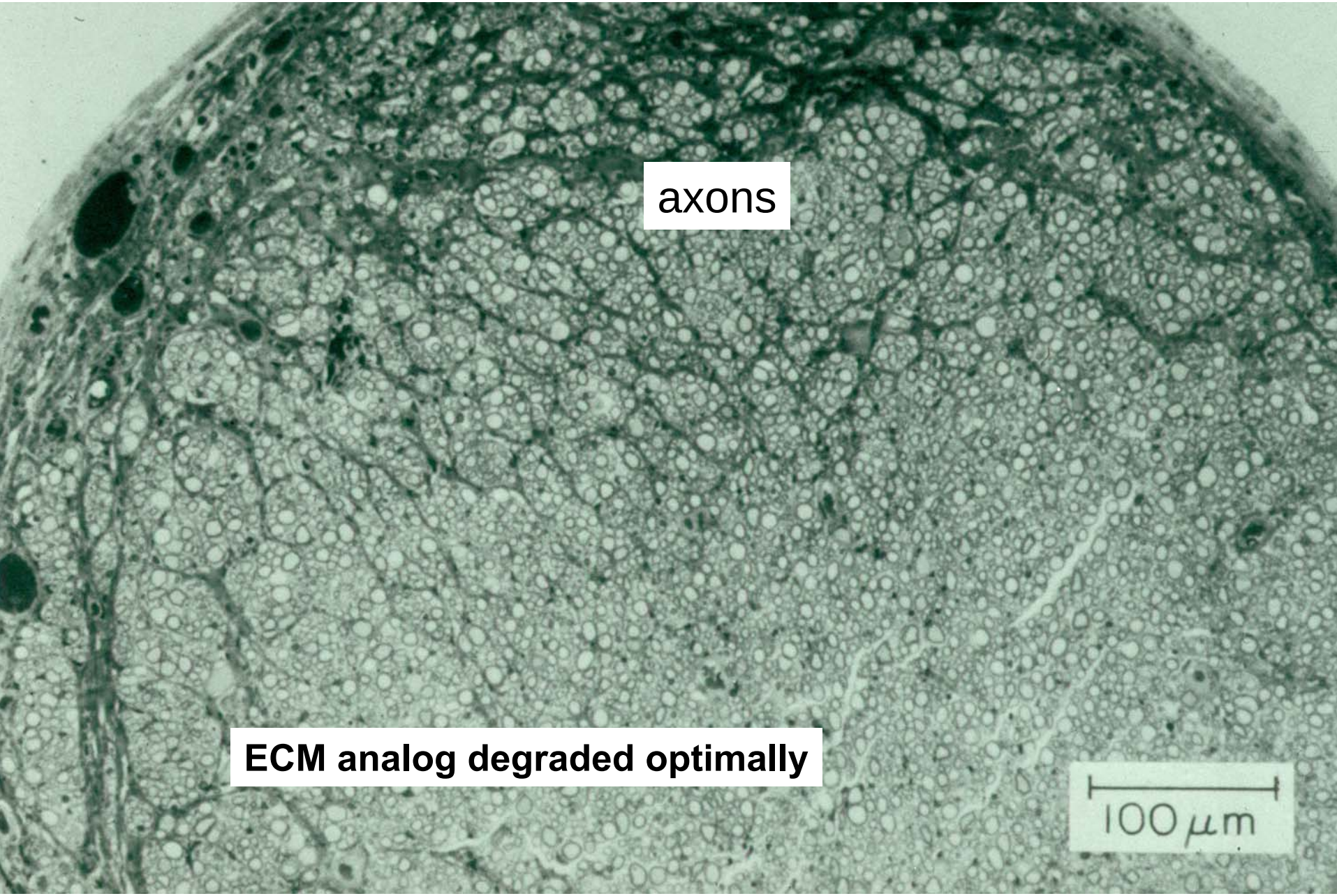
Photo removed due to copyright restrictions.

unfilled

Cross section-optical microscopy. Poorly regenerated nerve

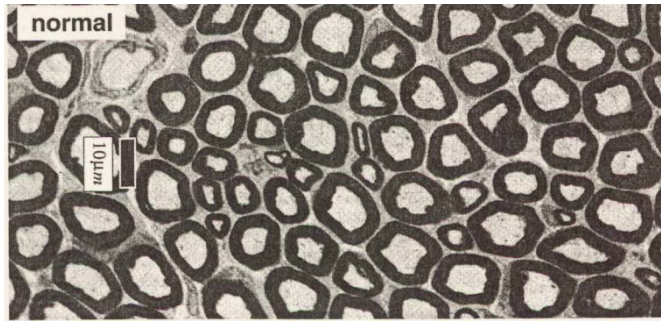


Cross section-optical microscopy. Well-regenerated nerve



Histomorphometry-cross sections of peripheral nerves regenerated using scaffolds with variable degradation rate

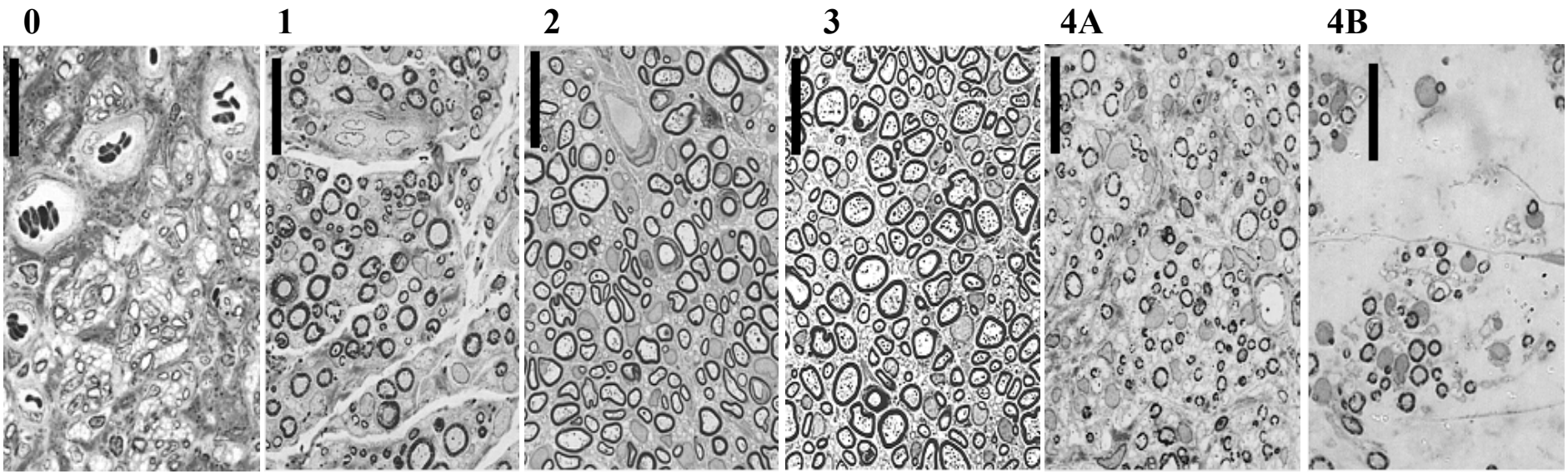
Normal Sciatic Nerve
(Chamberlain, 2000)



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 scaffold degradation rate

Problems and advantages of chemical symbolism

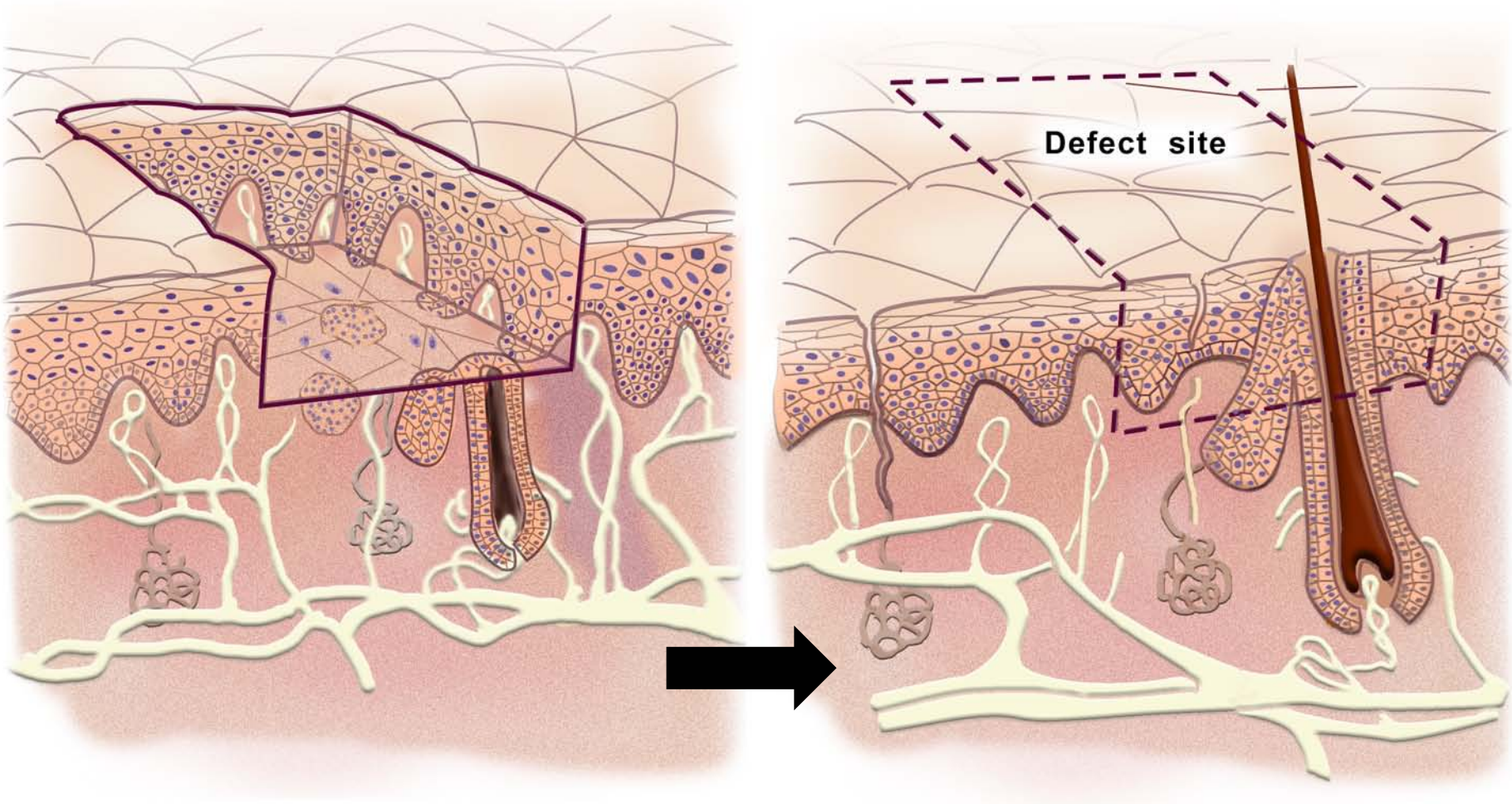
- **No stoichiometric data currently available! How many cells? What is concentration of cytokine X? Ligand density? Work with "Reaction diagrams", not chemical equations.**
- **Neither reactants nor products currently have standardized, time-invariant structure, as do chemical compounds.**
- **BUT gain rapid estimate of minimum requirements for synthesis of tissues and organs.**
- **Look for similarities between different organs (e.g., skin vs. nerves).**

B. The central question in organ synthesis

Which tissues in the triad do not regenerate spontaneously?

- When excised from an organ, the epithelia are regenerated spontaneously. Examples: the epidermis in skin, the myelin sheath in nerves.
- Likewise, the basement membrane regenerates spontaneously on the stroma.
- However, the stroma does not regenerate spontaneously. Examples: dermis in skin, endoneurium in nerves.

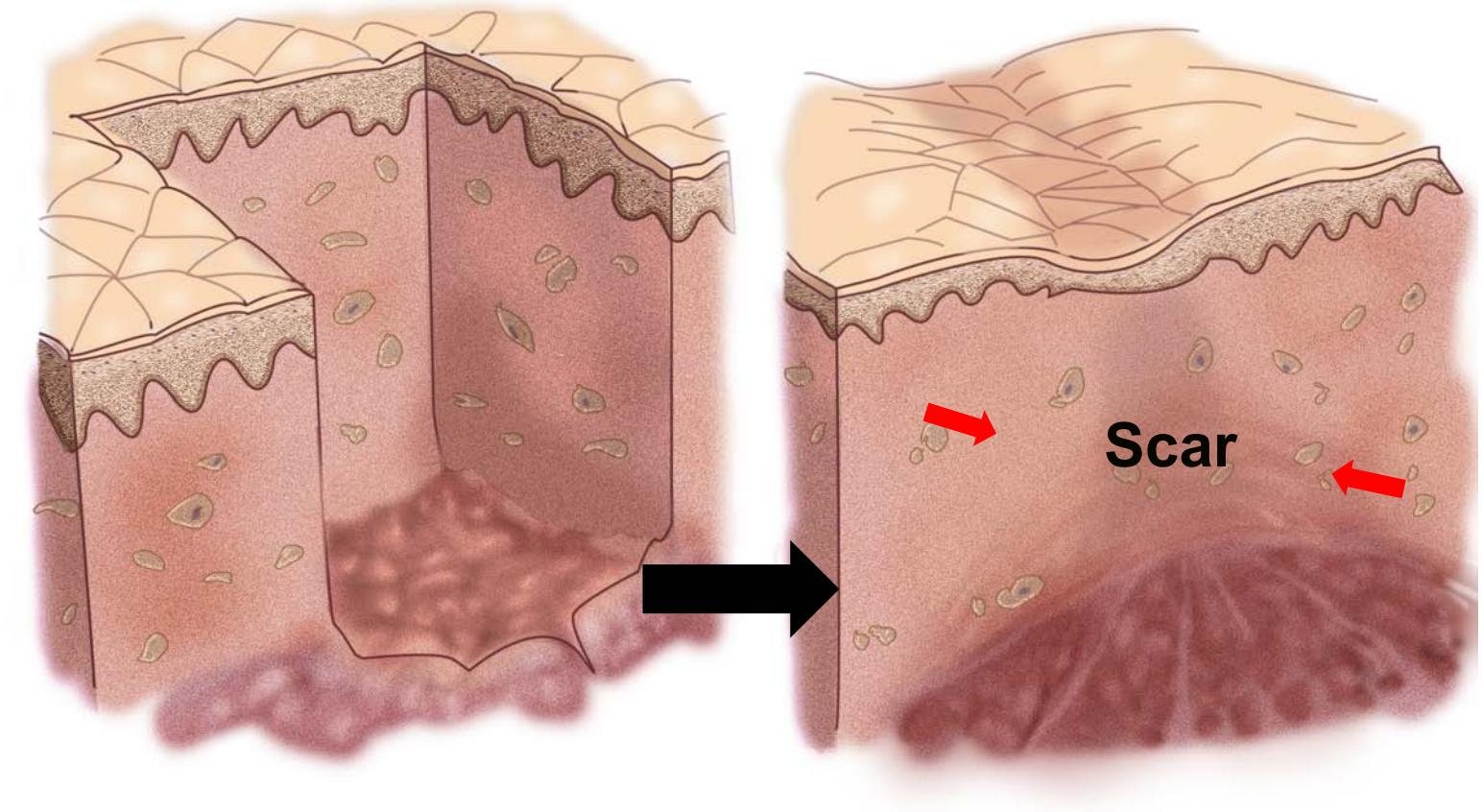
SKIN: The epidermis regenerates spontaneously



Epidermis lost. Dermis intact.

Spontaneous regeneration

SKIN: Scar formation. The dermis does not regenerate.

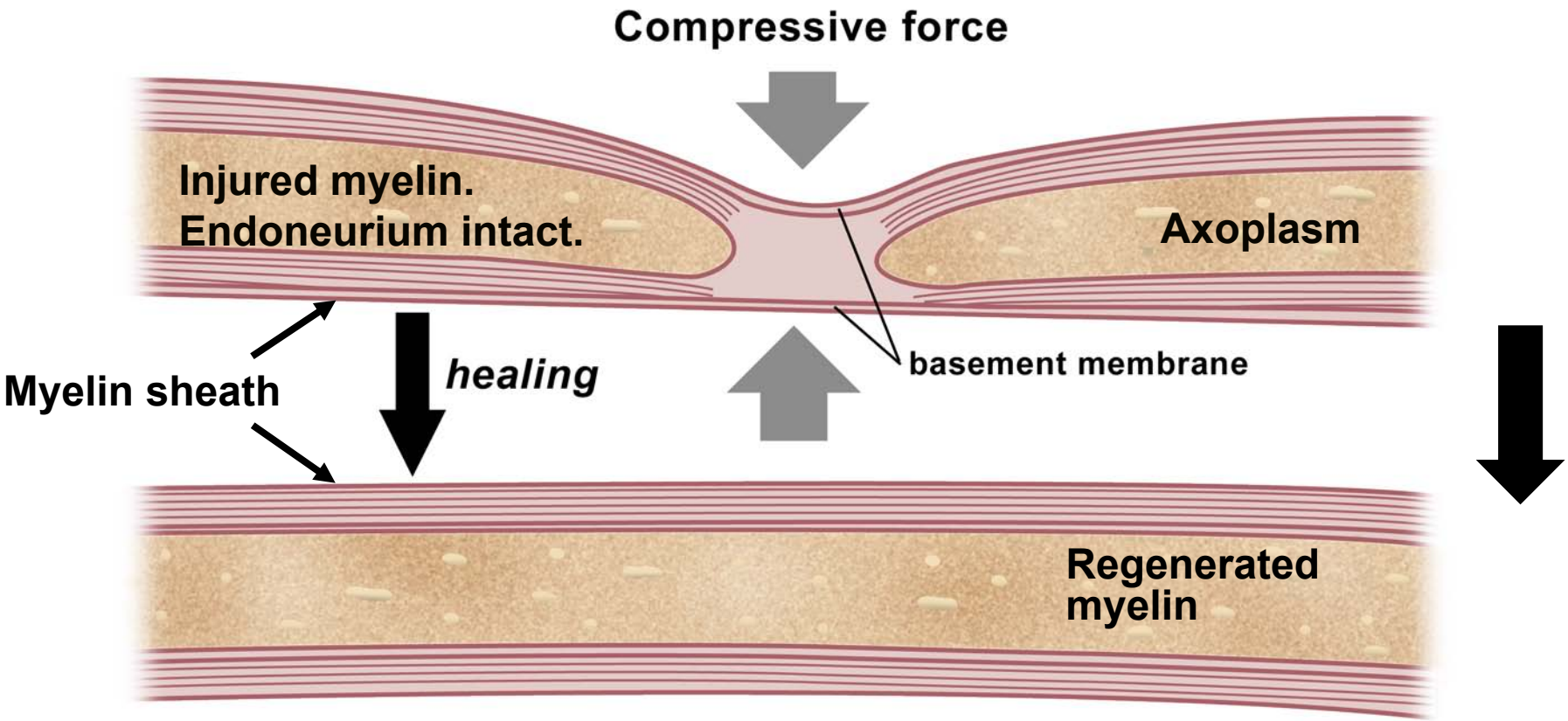


Figures by MIT OpenCourseWare.

Epidermis and dermis both lost to severe injury

Closure by contraction and scar formation

NERVE: The injured myelin sheath regenerates spontaneously



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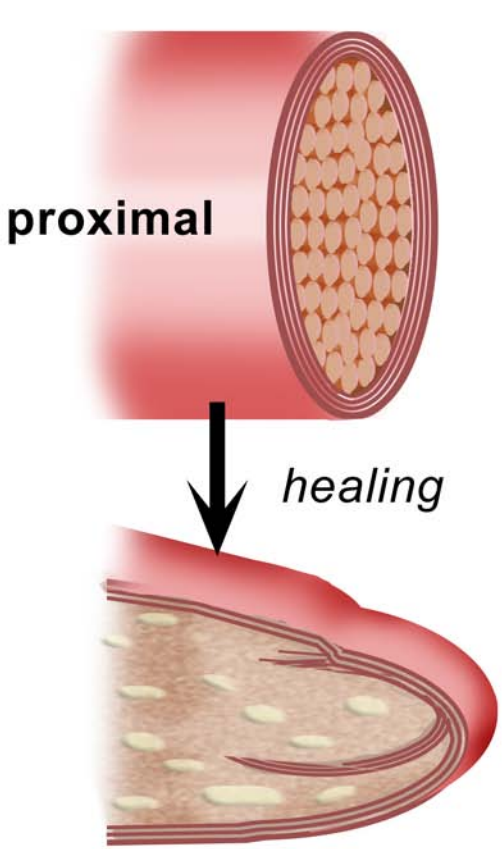
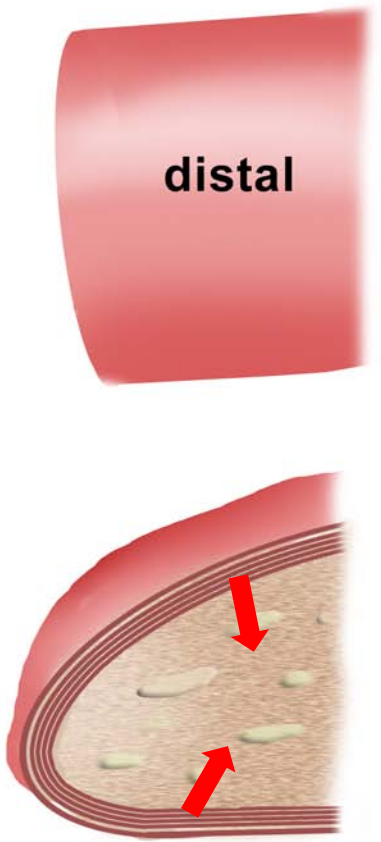
Neuroma formation. The endoneurium does not regenerate.



Transected nerve. Both myelin and endoneurium are severely injured.

Neuroma forms at each stump by contraction and scar formation.



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

Intact nerve fiber

Spontaneously healed nerve fiber (scar)

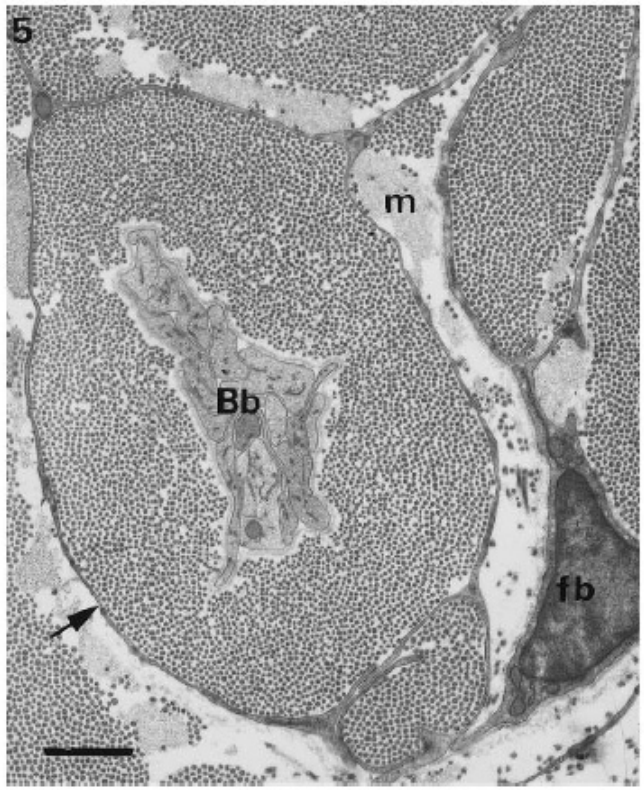


Fig. 5. Electron micrograph of a collagen domain containing a central Büngner band (Bb). The domain is encircled by thin fibroblast processes (arrow) which interdigitate in the upper right of the figure. These processes do not posses a basal laminal ensheathment whereas the fibroblast (fb) in the lower 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.

The central question is…

- **Epithelia and basement membrane (BM) are synthesized from remaining epithelial cells.**
- **The stroma is not synthesized from remaining stromal cells. Instead these cells induce closure of the injury by contraction and synthesis of scar.**
- ***Therefore, the central question in organ synthesis is how to synthesize the stroma.***
- **Once the stroma has been induced to synthesize, epithelial cells can spontaneously synthesize both epithelia and BM over it ("sequential" synthesis).**

C. What is required to synthesize an organ?

Required vs. redundant reactants

- **Investigators typically supply (add) reactants based on favored hypotheses. Often, reactants supplied are not required to synthesize tissue or organ.**
- ***In vitro* all reactants, including culture medium, are supplied by investigator.**
- ***In vivo* the reactor spontaneously supplies exudate that contains certain reactants (endogenous reactants). The investigator supplies other reactants (exogenous).**
- **What are the minimal reactants that suffice to synthesize a tissue or organ? These are the "required" reactants.**

Method used to identify required reactants

Collect data from over 70 groups of investigators of skin and peripheral nerve (Ch. 7). All worked with standardized reactors. Some worked in vitro with cells in culture; others in vivo with animals (e.g., rat, mouse)

Summarize complex protocol and results obtained by each investigator in the form of a "reaction diagram".

Omit some information. *In vitro* studies: Omit showing medium. *In vivo* studies: Do not show endogenous reactants; show only reactants that are supplied by investigator (exogenous).

Results.
Use color code for reactants

- epithelial cells (skin: keratinocytes, KC; nerve: Schwann cells, SC)
- stromal cells (fibroblasts, FB)
- matrices (analogs of extracellular matrix or synthetic polymers, CBL, DRT, COG, etc.)

Conventions used in reaction diagrams

1. **Epithelial cells are blue. Stromal cells are orange. Matrices are underlined in handout notes (but not in text).**
2. **Products are abbreviated (e.g., E = epidermis; E ·BM = epidermis with BM attached; E·BM·D = partial skin).**
3. **Reaction diagrams describe processes *in vitro* unless *in vivo* is specified over reaction arrow.**
4. **Complete tabulation of reaction diagrams and abbreviations in text pp. 194-197 (skin) and pp. 198-200 (nerve).**

Synthesis of an Epidermis (E)

- **epithelial cells: KC, SC**
- **stromal cells: FB**
- **matrix: DRT, CBL, L-DRT, COG etc.**

KC + FB → E	KC + CBL → E
KC → E (simplest is bold-fonted)	KC + FB + L-DRT → E
KC + DRT → E	KC + FB + COG → E

Synthesis of a basement membrane (E·BM)

KC + COG → E·BM	**KC → E·BM (simplest)**
KC + CBL → E·BM	KC + FB + PGL → E·BM(?) (in vivo)
KC → E·BM (in vivo)	KC + FB + COG → E·BM
KC + FB + COG → E·BM	KC + COFL → No BM
KC + FB + L-DRT → E·BM (in vivo)	KC + PL → No BM
KC + FB + NY → E·BM	KC + DRT → E·BM (in vivo)

Synthesis of a dermis (D)

DRT → D (in vivo) (simplest)	KC → No D (in vivo)
KC + FB + COG → No D	KC + FB + L-DRT → D (in vivo)
KC + FB + COG → D (in vivo)	KC + FB + L-DRT → No D
KC + CBL → No D	KC + FB + PGL → No D
KC + DRT → No D	KC + FB + PGL → D (in vivo)

Synthesis of skin (partial skin = PS = E·BM·D)

KC + FB + COG → PS (in vivo)	KC + CBL → PS (in vivo)
KC + DRT → PS (in vivo) (simplest)	KC + FB + PGL → PS (in vivo)
KC + FB + L-DRT → PS (in vivo)	

Select simplest routes for skin synthesis

- Epidermis: KC → E
- Basement Membrane: KC → E·BM
- Dermis: DRT → D (in vivo)
- Skin (partial): KC + DRT → PS (in vivo)

- Exogenous fibroblasts not required.
- Exogenous cytokines not required.
- Epithelia and BM synthesized in vitro. Dermis synthesized in vivo.
- Partial skin synthesized in vivo.

Sequential vs. simultaneous synthesis of skin tissues

A. Sequential (two-step) synthesis:

1. Synthesize the dermis using a template.

DRT → D

2. Epidermis and BM later spontaneously synthesized by residual epithelial cells.

KC → E·BM

B. Simultaneous (one-step) synthesis of dermis and epidermis:

Seed template with epithelial cells.

KC + DRT → E·BM·D = PS

Simplest routes for nerve synthesis

- **Myelin sheath: SC → MAX**
- **Basement membrane: SC → MAX·BM**
- **Endoneurium: silicone tube → ED(?)**
- **Conducting nerve trunk: various tubes → MAX·BM·ED(?)·PN**

- **Exogenous fibroblasts not required to be added.**
- **Exogenous cytokines not required to be added.**
- **Epithelia and BM synthesized in vitro.**
- **Endoneurium uncertain. Nerve trunk synthesized in vivo.**

D. Trans-organ reaction diagrams

Select only the simplest

	EPITHELIA (in vitro)	BM (in vitro)
skin	KC → E	KC → E·BM
nerve	SC → MAX	SC → MAX·BM
	STROMA (in vivo)	**ORGAN (in vivo)**
skin	DRT → D	DRT → PS
nerve	tubes → ED(?)	tubes → nerve trunk

Summary of trans-organ rules for organ synthesis

What are the similarities between the simplest pathways required to synthesize skin and peripheral nerves?

Both in skin and peripheral nerve:

- **Synthesis of epithelia simply required supply of epithelial cells in vitro (appropriate medium also required).**
- **Synthesis of stroma required supply only of an appropriate scaffold in vivo.**

Various synthetic routes

Route 1: Sequential synthesis

Stroma synthesized first using appropriate matrix (regeneration template). Epithelia and basement membrane both synthesized spontaneously later on the new stroma by endogenous epithelial cells.

Route 2: Simultaneous synthesis

All three tissues can be simultaneously synthesized using template seeded with epithelial cells.

Route 3: Modular organ synthesis? Synthesize each tissue in separate reactor, then combine.

Summary of synthetic rules for tissues and organs

1. Use symbolism of organic chemistry to compare several independent synthetic protocols from literature.
2. The central problem is synthesis of stroma.
3. Epithelial cells and appropriate medium only required to synthesize epithelia in vitro. Appropriate matrix only must be supplied to synthesize stroma in vivo.
4. Applicability to other organs?

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

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