Harvard-MIT Division of Health Sciences and Technology
HST.535: Principles and Practice of Tissue Engineering
Instructor: I. V. Yannas

Collagen-GAG scaffolds for organ regeneration processes

Prof. I. V. Yannas

MIT

Reference: I.V.Yannas, *Tissue and Organ Regeneration in Adults*, Springer, 2001

Analogs of extracellular matrix

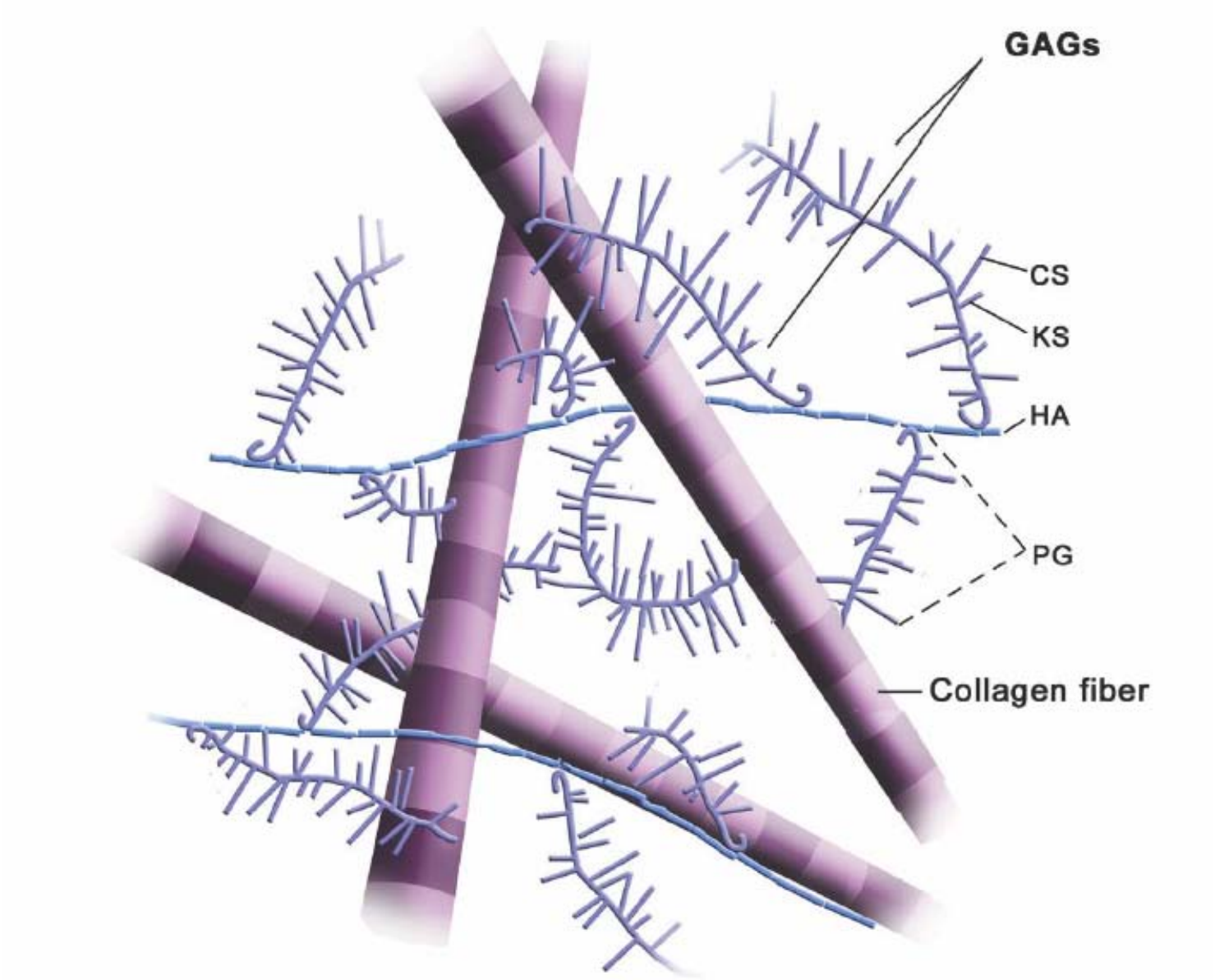


Figure by MIT OCW. After Ricci.

Question to answer:
Why use collagen-GAG scaffolds to induce organ regeneration?

Study requirements for organ synthesis in vivo. Use chemical symbolism to simplify analysis.

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)

Problems and advantages of chemical symbolism

- **No stoichiometric data currently available! How many cells? What is concentration of cytokine X? Ligand density? "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).**

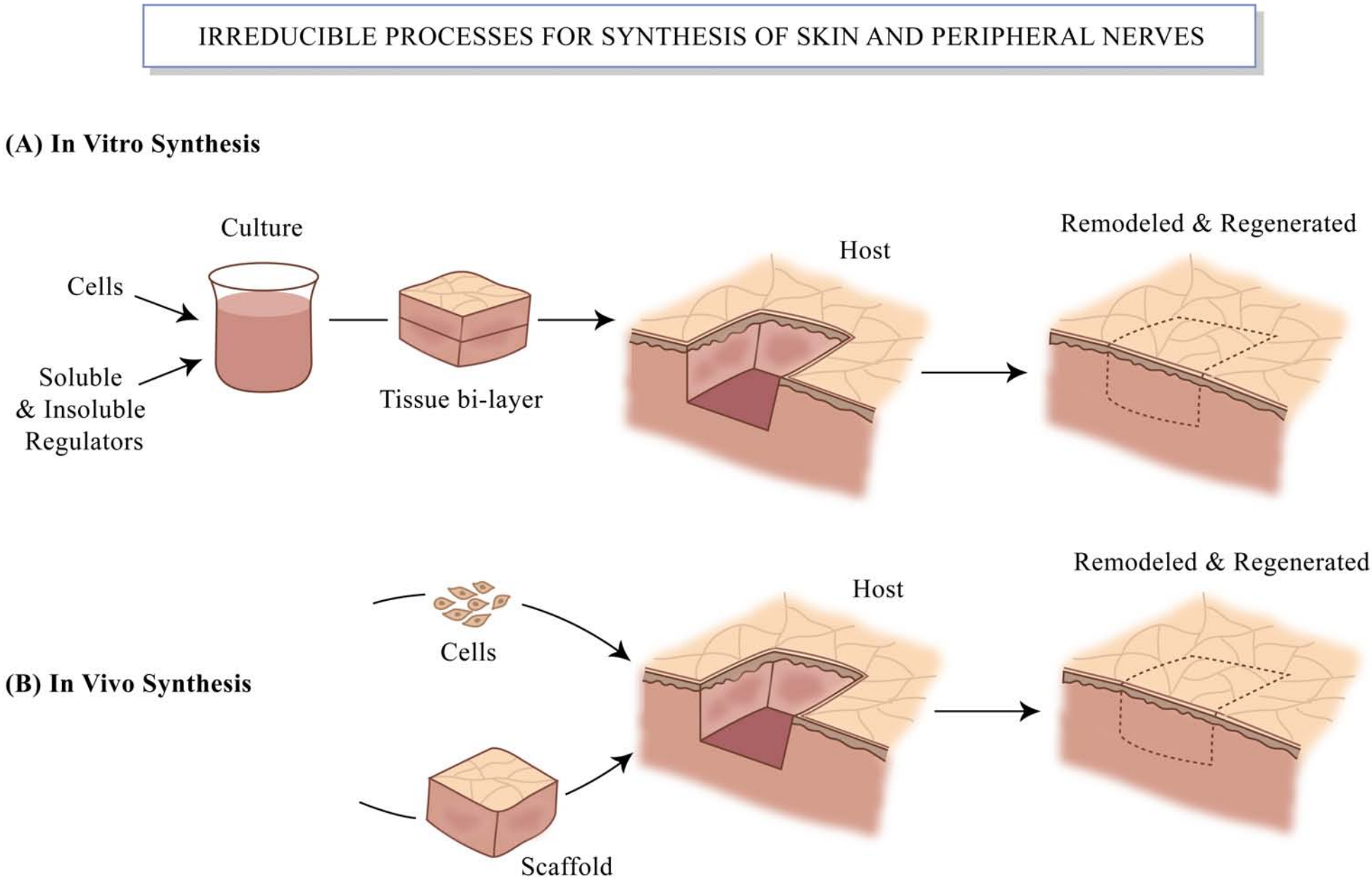
Transition to biology
I. Reactants

- **Cells migrate, proliferate, synthesize matrices and cytokines, degrade matrices, etc.**
- **Cytokines and growth factors 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").**

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?



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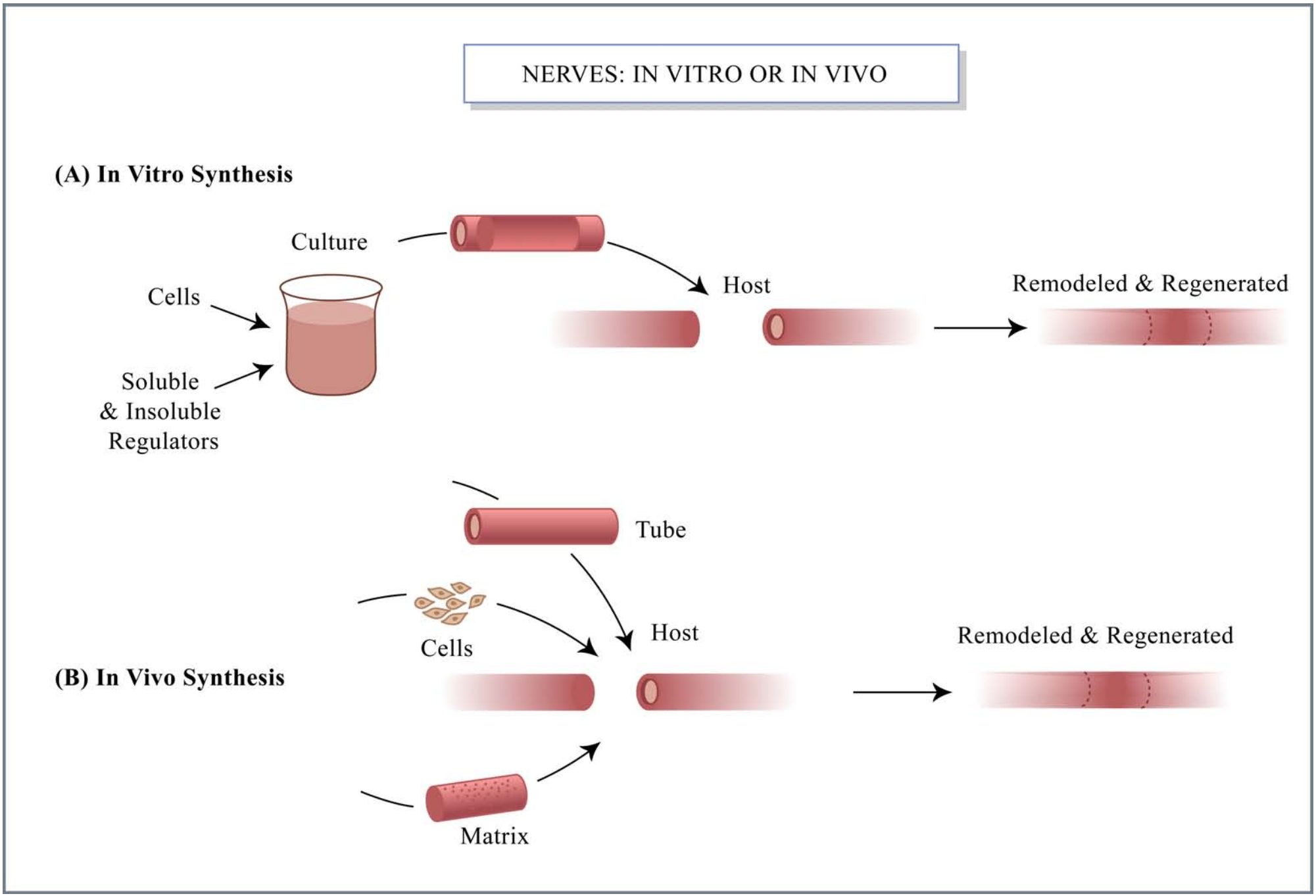
Nerves: In vitro or in vivo?



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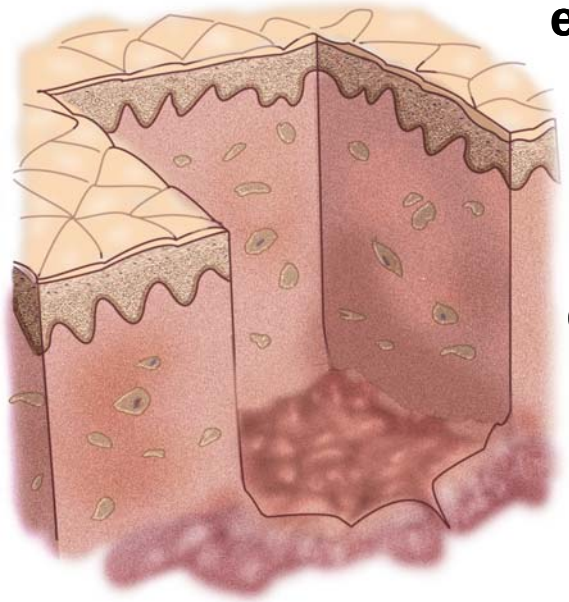
Standardized reactors

SKIN



PERIPHERAL NERVE

Figures by MIT OCW.

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).

Members of the tissue triad

- **EPITHELIA**

  100% cells. No matrix. No blood vessels.

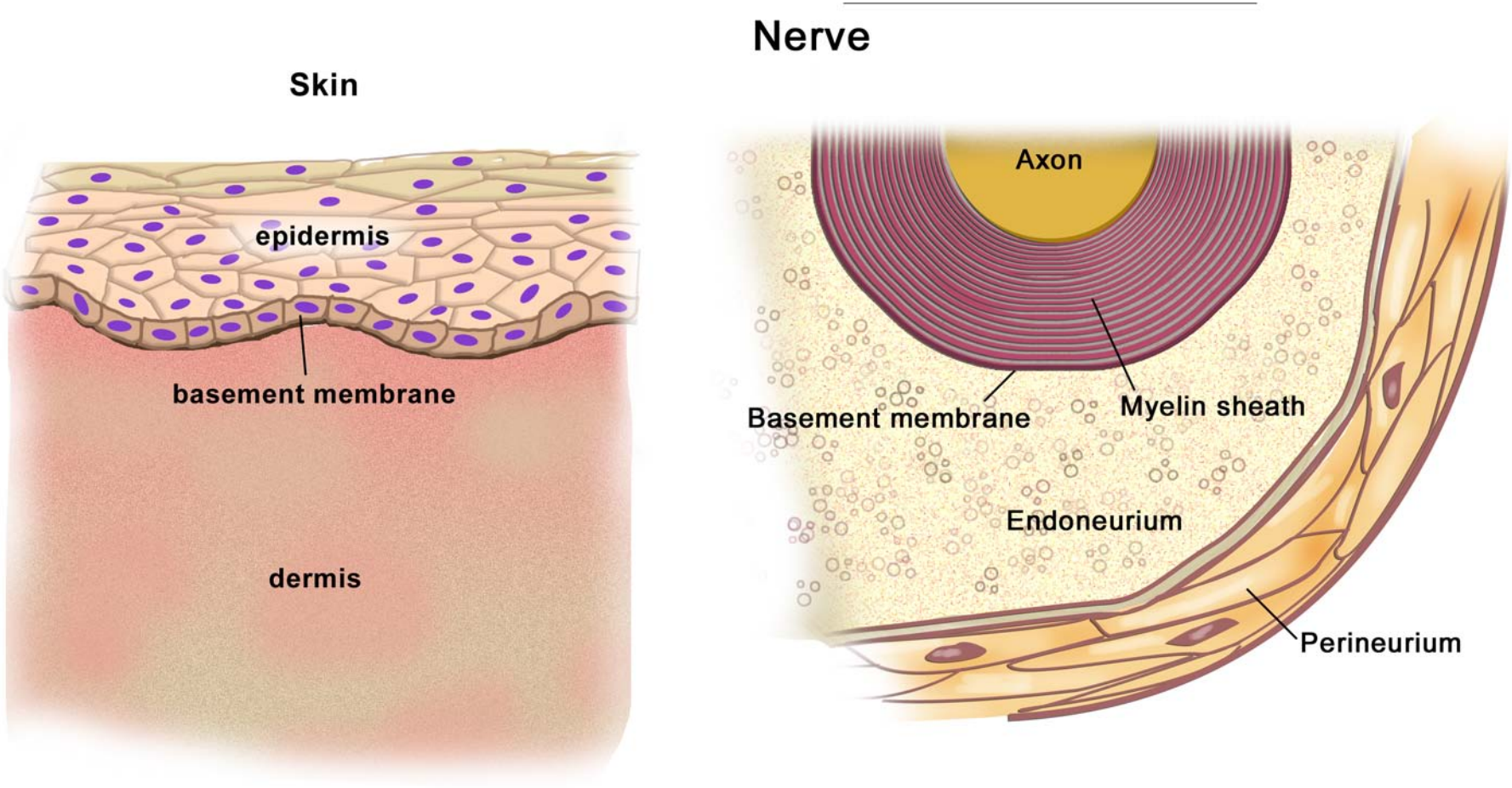
- **BASEMENT MEMBRANE**

  No cells. 100% matrix. No blood vessels.

- **STROMA (CONNECTIVE TISSUE)**

  Cells. Matrix. Blood vessels.

The tissue triad in skin and nerves



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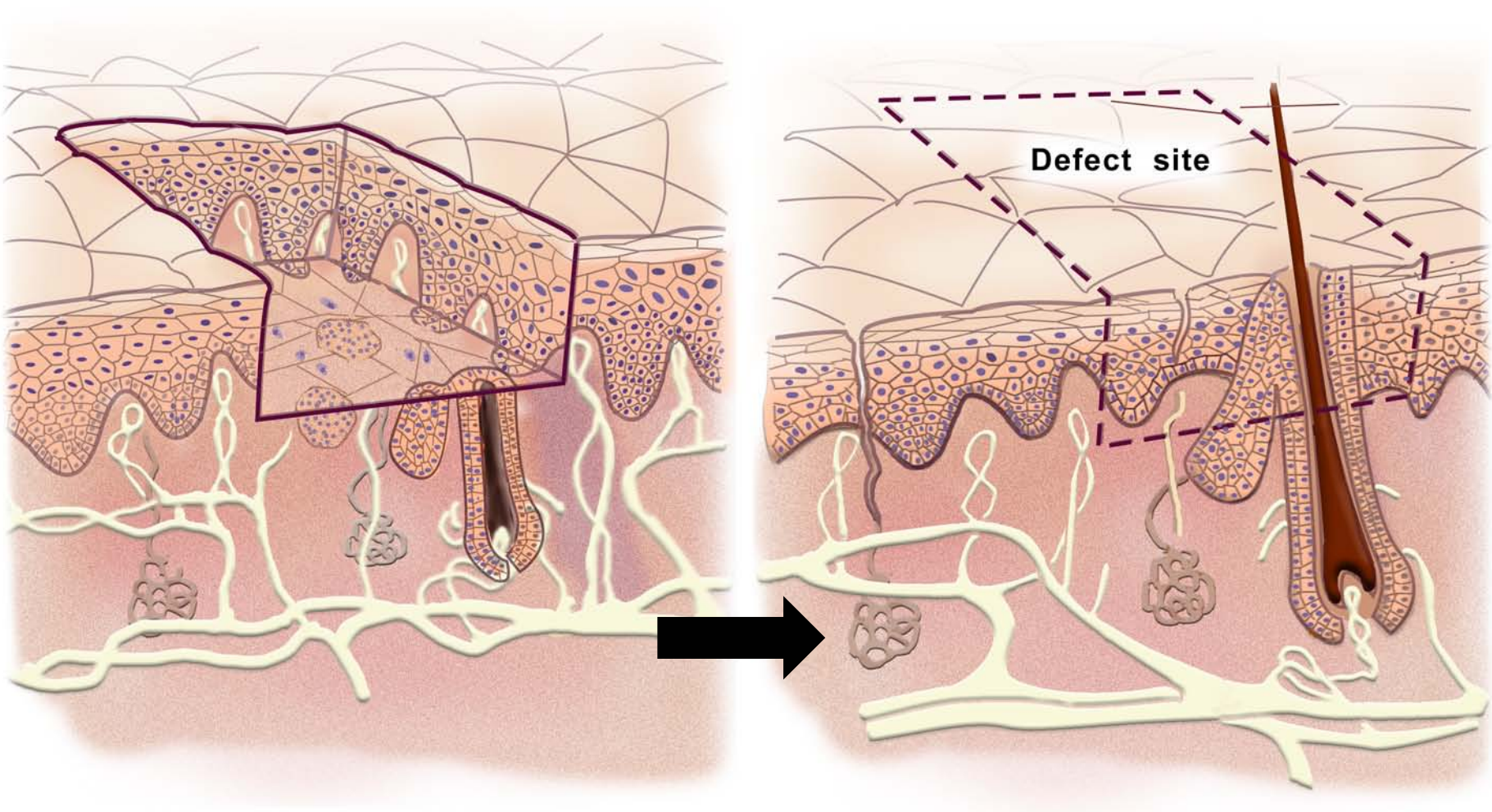
The central question in organ synthesis

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.

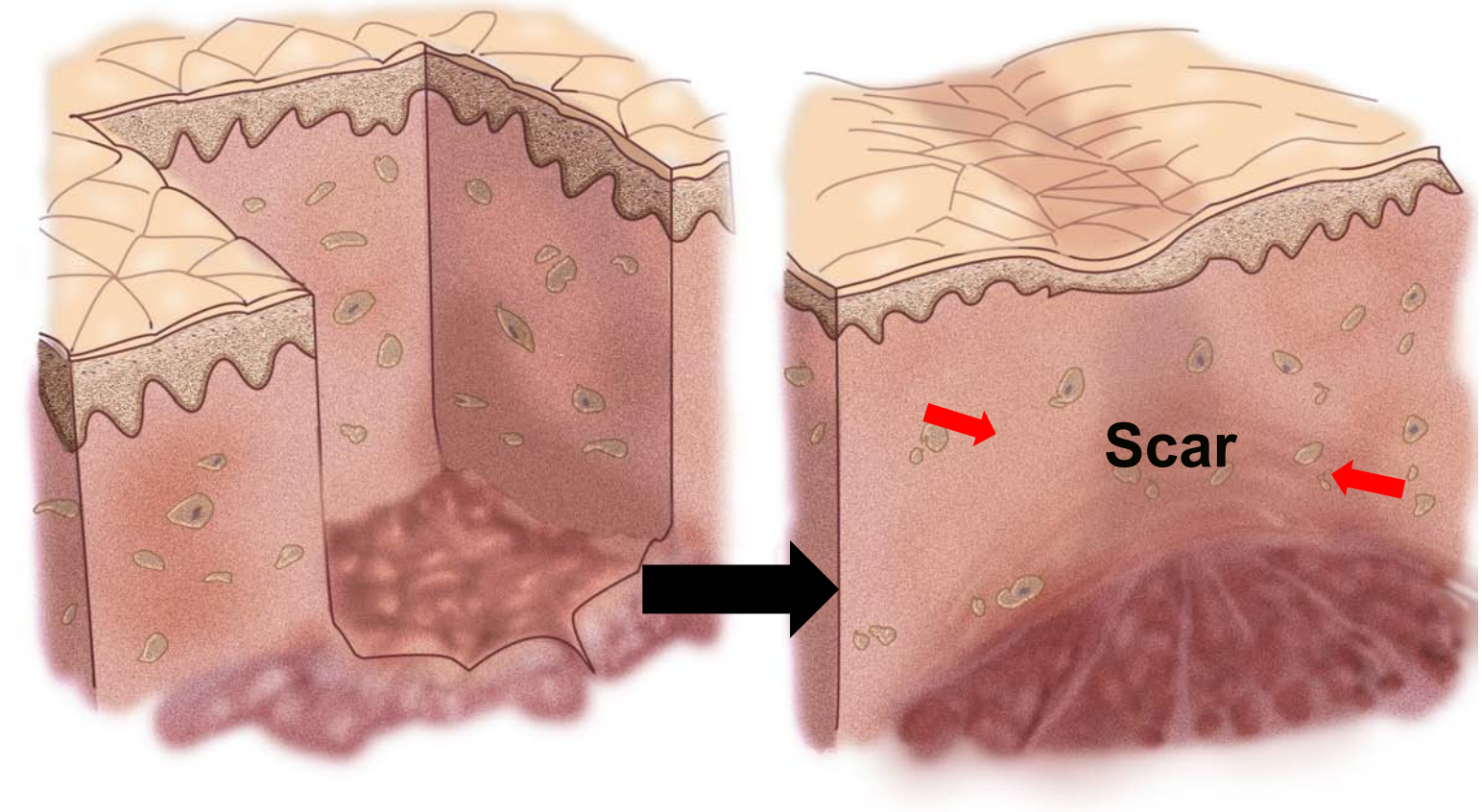
SKIN: The epidermis regenerates spontaneously



Epidermis lost. Dermis intact. **Spontaneous regeneration**

Figure by MIT OCW.

SKIN: Scar formation. The dermis does not regenerate.



Epidermis and dermis both lost to severe injury

Closure by contraction and scar formation

Figure by MIT OCW.

NERVE: The injured myelin sheath regenerates spontaneously

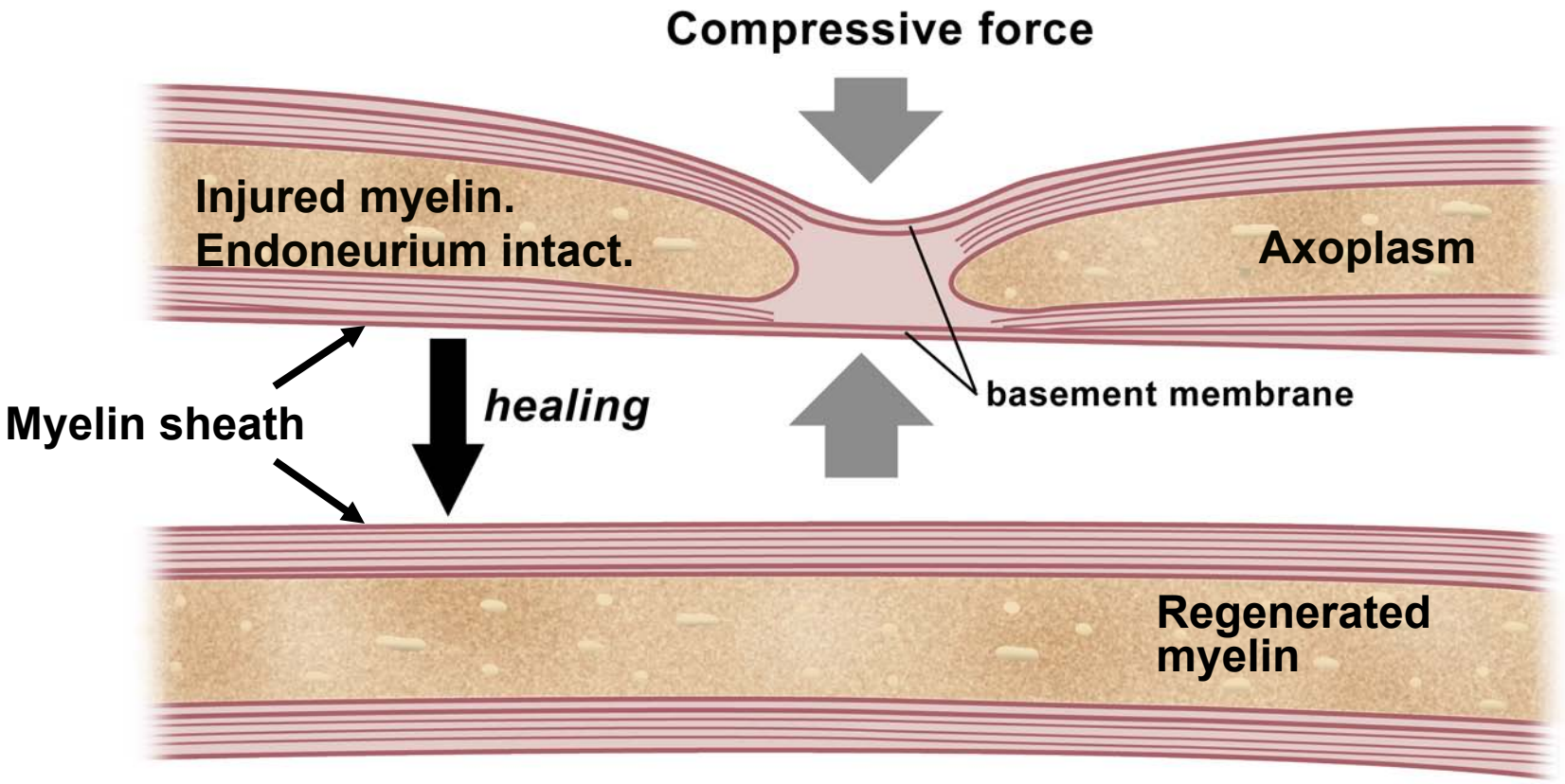
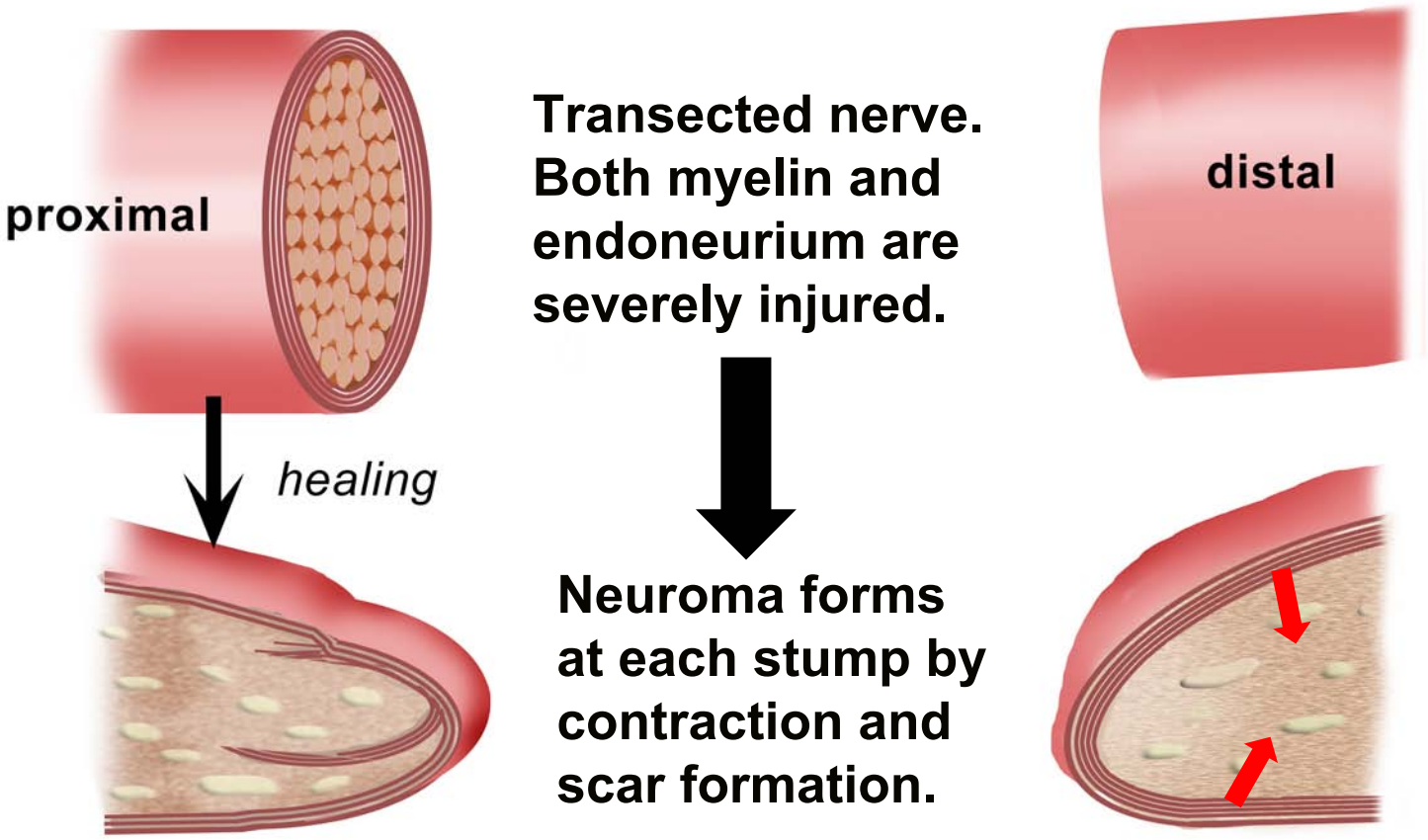


Figure by MIT OCW.

Neuroma (scar) formation. The endoneurium (stroma) does not regenerate.



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Intact nerve fiber

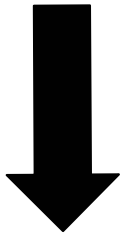

Photo removed for copyright reasons.
See Figure 2.5 in Yannas, I. V.
Tissue and Organ Regeneration in Adults.
New York: Springer, 2001. ISBN: 0387952144.

Spontaneously healed nerve fiber (scar)

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).**

Which reactants are required to be supplied by the investigator to synthesize an organ?

Use empirical trans-organ rules to find requirements for addition of cells, scaffolds and growth factors

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.**

Finally answer question:
Why use collagen-GAG scaffolds to induce organ regeneration?

Based on data from synthesis of skin and peripheral nerves :

- **Synthesis of epithelia can be accomplished in vitro (does not require in vivo environment). It simply requires supply of epithelial cells and culture medium. Scaffold not required.**
- **Synthesis of stroma has only been accomplished in vivo. It requires supply only of an appropriate scaffold. Addition of stromal cells (e.g., fibroblasts) or growth factors (e.g., TGF-β, PDGF) is not required.**
- **An appropriate scaffold is required for organ synthesis. Epithelial cells speed up synthesis. Stromal cells (e.g., fibroblasts) or growth factors need not be added.**

Various synthetic routes

Route 1: Sequential synthesis

Stroma synthesized first using appropriate matrix (regeneration template). Epithelia and basement membrane both synthesized spontaneously later in contact with 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.

Synthesis of active ECM analogs:

--- Ionic complexation of collagen/GAG.

--- Formation of pore structure.

--- Crosslinking.

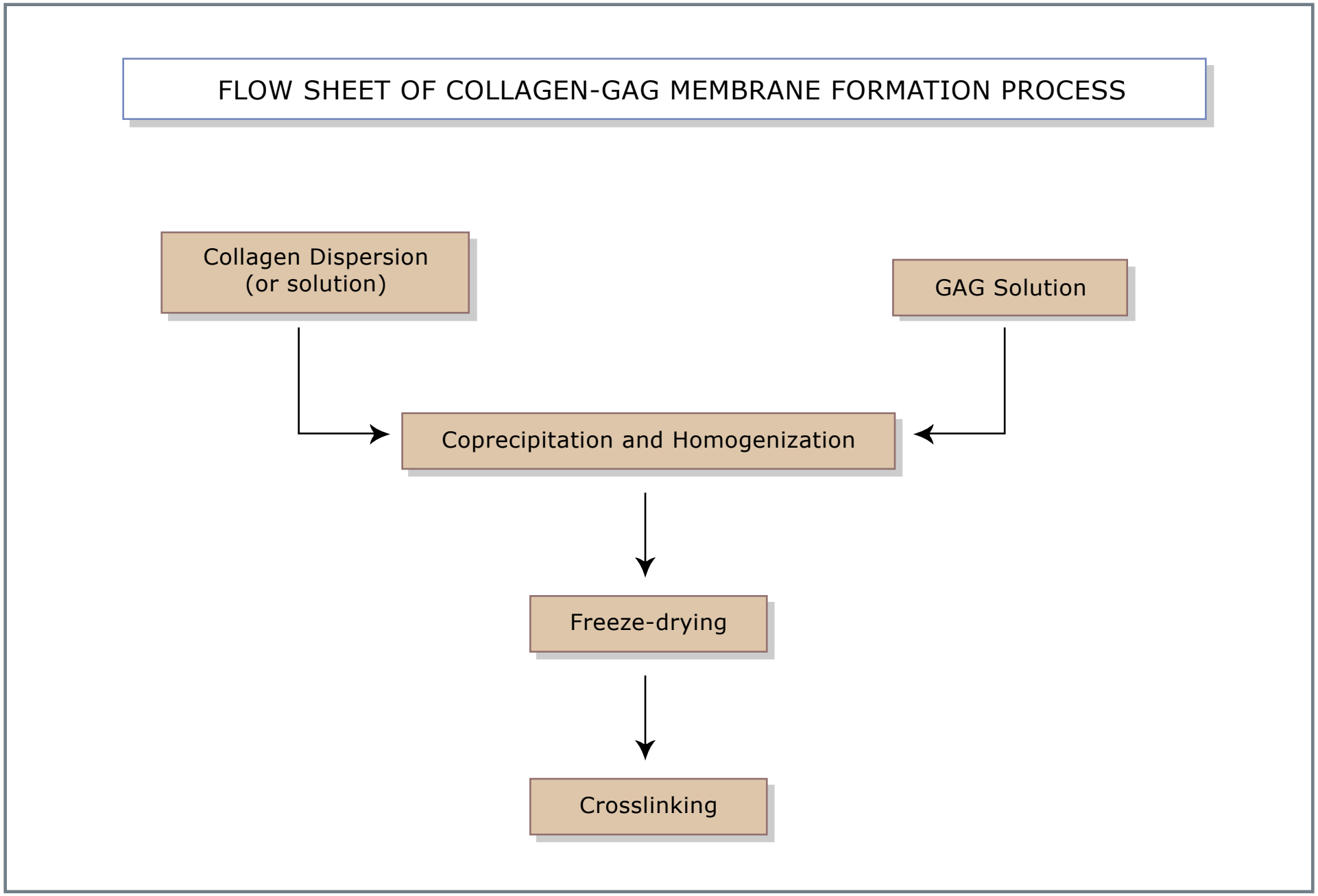

Figure by MIT OCW.

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Which collagen-GAG scaffolds are biologically active as regeneration templates?

Critical Structural Feature	Role in regeneration
A. SKIN	
Chem. Composition >2% GAG Deleted collagen quaternary structure	Ligand identity Downregulation of contractile cells
Pore diameter 20—120 μm	Ligand density
Degradation half-life 10-15 d	Duration of ligands
B. NERVE	
Chem. Composition Deleted collagen quaternary structure	[not studied] [not studied]
Pore diameter ~ 5 μm	Ligand density
Degradation half-life ~ 1-10 wk	Duration of ligands

Mechanism of regenerative activity of collagen-GAG scaffolds

1. Certain ECM analogs are biologically active scaffolds (regeneration templates) that induce regeneration of tissues and organs: skin, peripheral nerve and the conjunctiva (eye) in humans and experimental animals.
2. Regeneration templates lose their activity if the following structural features fall outside a narrow range: chemical composition, collagen quaternary structure, pore diameter, degradation rate.
3. The data suggest that templates induce regeneration in a defect by blocking selectively the contraction process that leads to closure of the defect in adults.
4. Templates block contraction by two basic mechanisms. First, by downregulating differentiation of fibroblasts to myofibroblasts. Second, by binding most of the contractile cells in the defect over a period corresponding to the duration of contraction in that defect. Binding requires the presence of appropriate ligands (chem. composition) at a minimal density (pore diameter) over a critical duration (degradation rate).

Summary

1. Of three types of tissue in an organ only stroma fails to regenerate spontaneously and needs to be induced to synthesize. Epithelia and basement membrane regenerate spontaneously.
2. There are three classes of “reactants”, i.e., cells, scaffolds and growth factors. Of these only an appropriate scaffold must be added to synthesize the stroma.
3. The appropriate scaffold (regeneration template) is synthesized to have the composition of an analog of the extracellular matrix, pore size within a critically defined range and degradation rate that matches the rate of tissue synthesis at the organ site.