Harvard-MIT Division of Health Sciences and Technology
HST.535: Principles and Practice of Tissue Engineering
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Facts and theories of organ regeneration in adults

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Outline

A. Introduction: Synthesis of organs, in vitro or in vivo?

B. Facts: Irreversible organ injury.

C. Facts: Antagonistic relation between contraction and regeneration.

D. Facts: Isomorphous replacement.

E. Theories. 1. Immunocompetence theory. 2. Contraction blockade + isomorphous replacement.

A. Introduction: Synthesis of organs, in vitro or in vivo?

Skin: In vitro or in vivo synthesis?

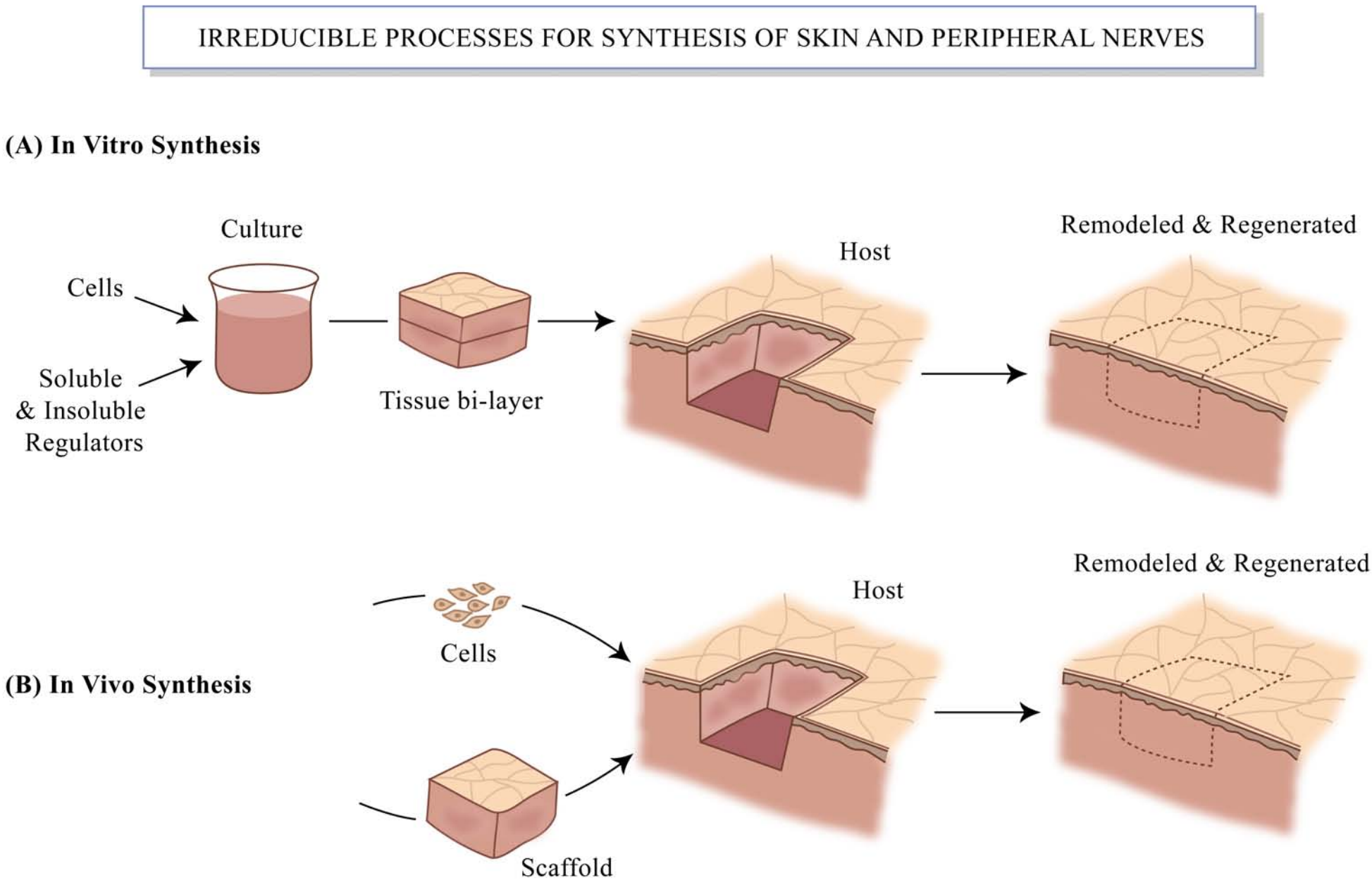


Figure by MIT OCW.

Peripheral nerves: In vitro or in

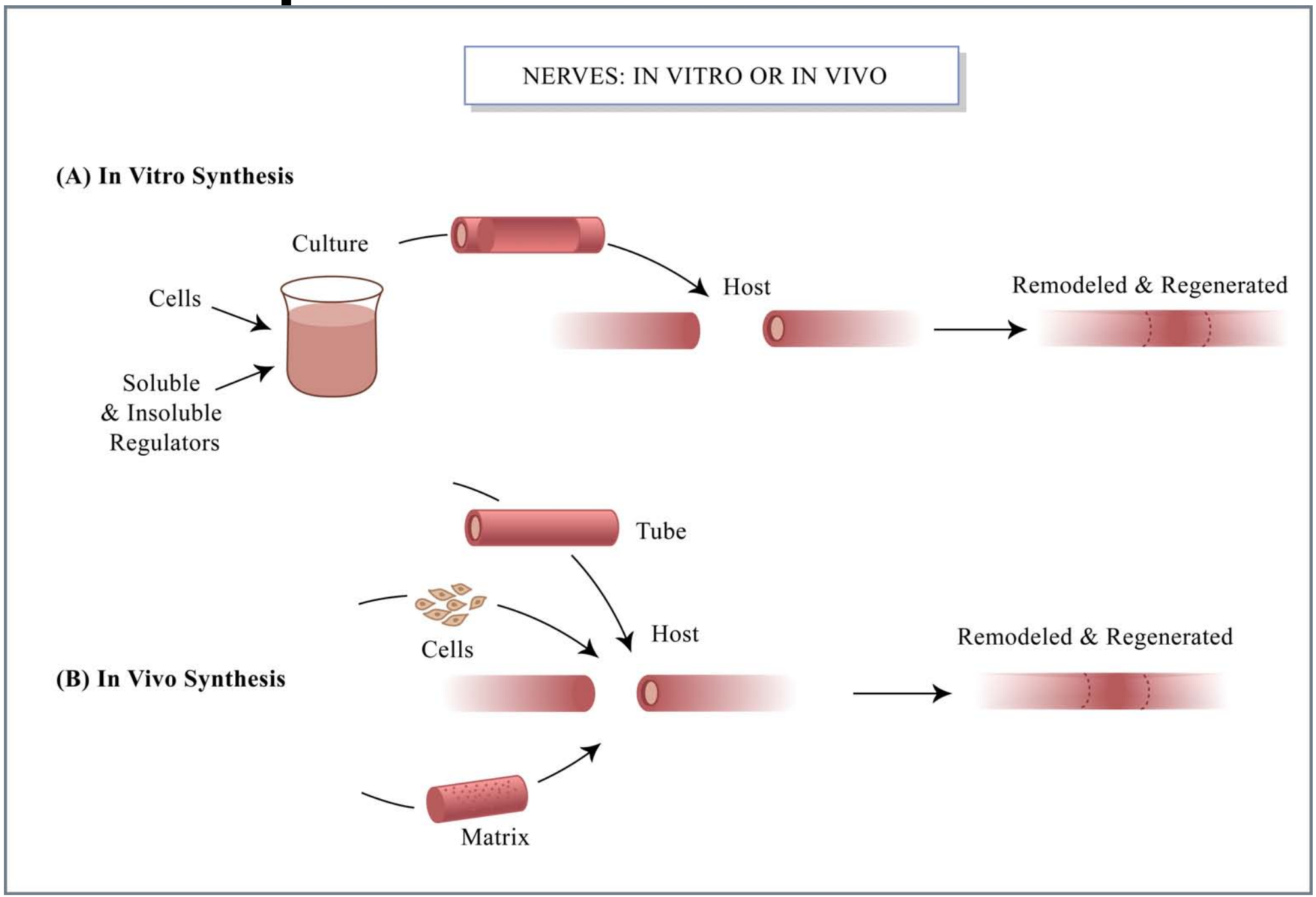


Figure by MIT OCW.

In vitro or in vivo?

Two published protocols, A and B, for synthesis of skin

A. **First step is *In vitro*: Keratinocytes + Fibroblasts + Collagen gel → Implant**
   Second step is *In vivo*: Implant → Skin

B. **Directly *In vivo*: Keratinocytes + Dermis regeneration template → Skin**

Direct *In vivo* synthesis is simpler:

- **Investigator focuses on one reactor only.**
- **Uses the endogenous cytokine field* and endogenous FB. No need to add growth factors, including angiogenesis factors.**

***Cytokine field: The unknown time- and space-dependent concentrations of growth factors and other cytokines in injured site.**

B. Irreversible organ injury.

Why study the healing process?

1. In vitro or in vivo method → implant

2. Implant → **injured anatomical site undergoing healing**

3. Implant + healing → organ synthesis

Two adult healing modes

Spontaneous healing in adults

injury → contraction + scar formation

Healing by regeneration in adults

injury → implant an active cell-seeded scaffold → MECHANISM? → organ synthesis

Reversible injury in an amphibian

Diagram removed for copyright reasons.
See Figure 1.1 in Yannas, I. V.
Tissue and Organ Regeneration in Adults.
New York: Springer, 2001. ISBN: 0387952144.

Spontaneous regeneration of amputated limb in the newt occurs independently of severity of injury

Goss, 1992

Irreversible injury in adult mammal

Photo removed
for copyright
reasons.

Burn victim suffering from severe contraction and scar formation

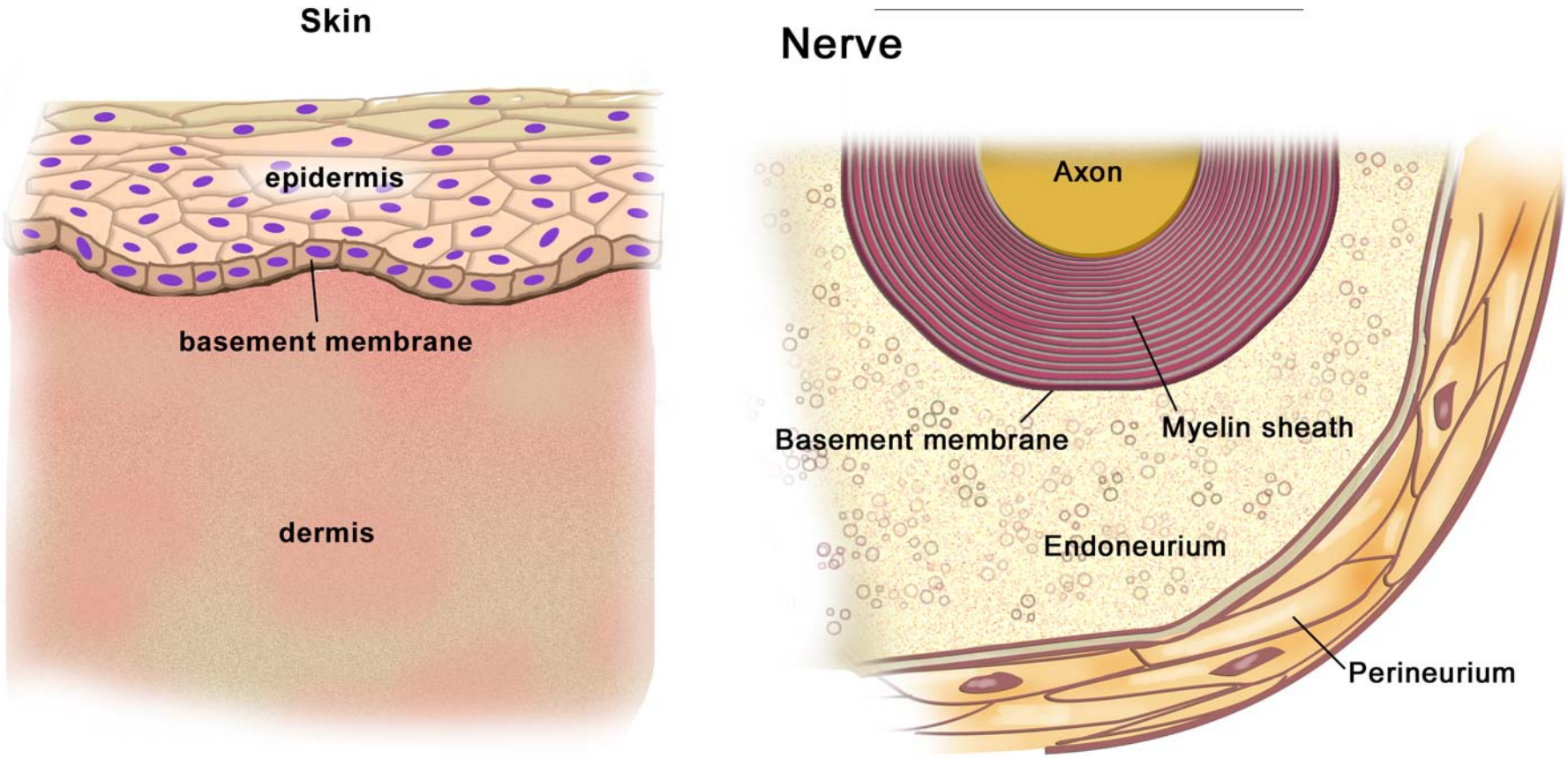
Tomasek et al., 2000

The tissue triad in skin and nerves

epithelial tissue: 100% cellular, no ECM

basement membrane: 100% ECM , no cells

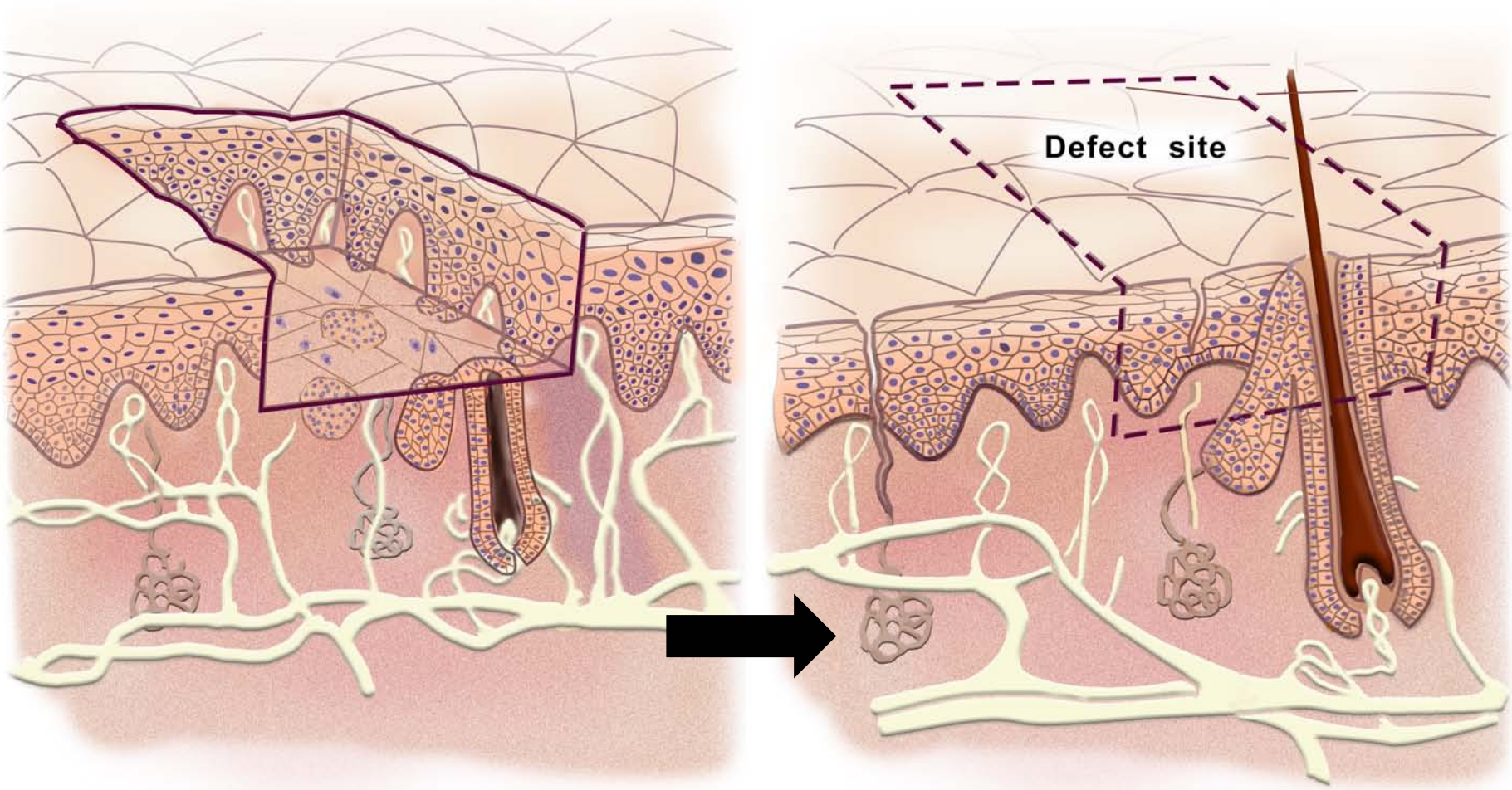
stroma: cells, ECM, blood vessels



Figures by MIT OCW.

Yannas, 2001

Skin: reversible injury



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

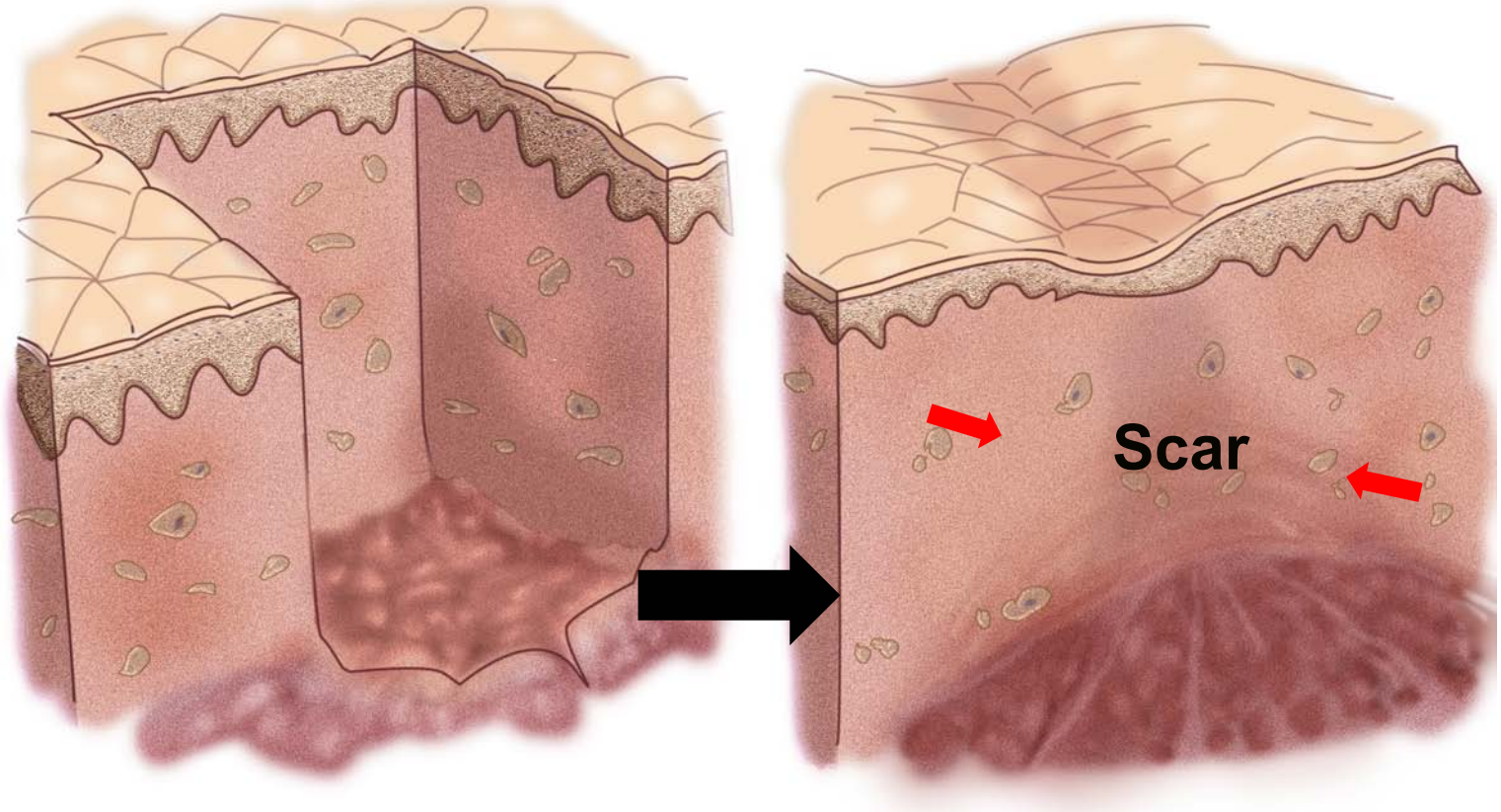
Figure by MIT OCW.

The epidermis is a regenerative tissue. After excision, it regenerates spontaneously. Reversible injury. No contraction. No scar.

Yannas, 2001

Skin: Irreversible injury

spontaneous healing of ful thickness skin excision by contraction and scar formation



Epidermis and dermis both lost to severe injury

Closure by contraction and scar formation

Figure by MIT OCW.

The dermis is a nonregenerative tissue in the adult. After excision, it does not regenerate spontaneously. Irreversible injury. Closes with contraction and scar formation.

Yannas, 2001

Peripheral nerve: reversible iniurv

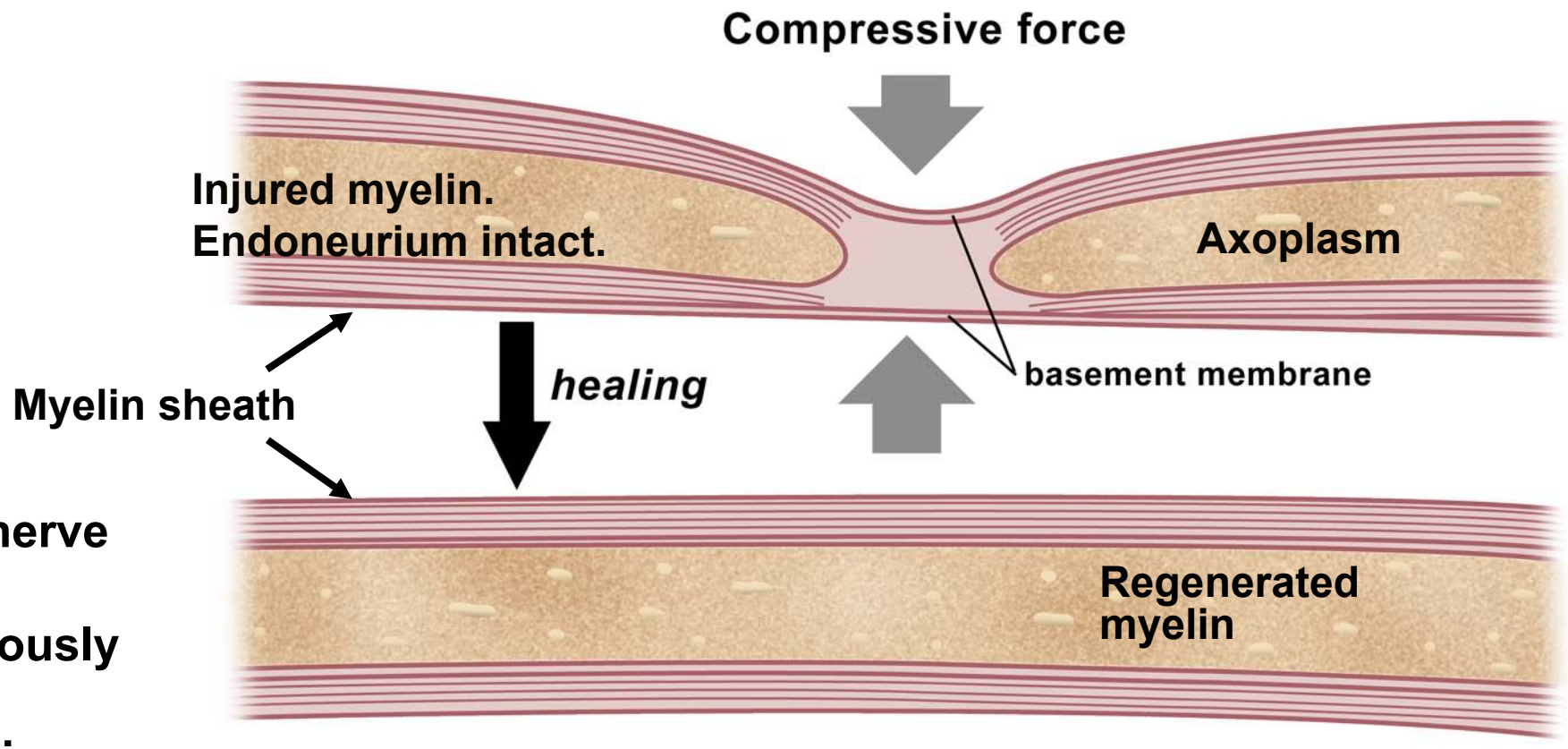


Figure by MIT OCW.

The myelin sheath is a regenerative tissue. Following nerve crushing with myelin disruption, the myelin regenerates spontaneously. Reversible injury. No contraction. No scar.

Yannas, 2001

Peripheral nerve: irreversible injury

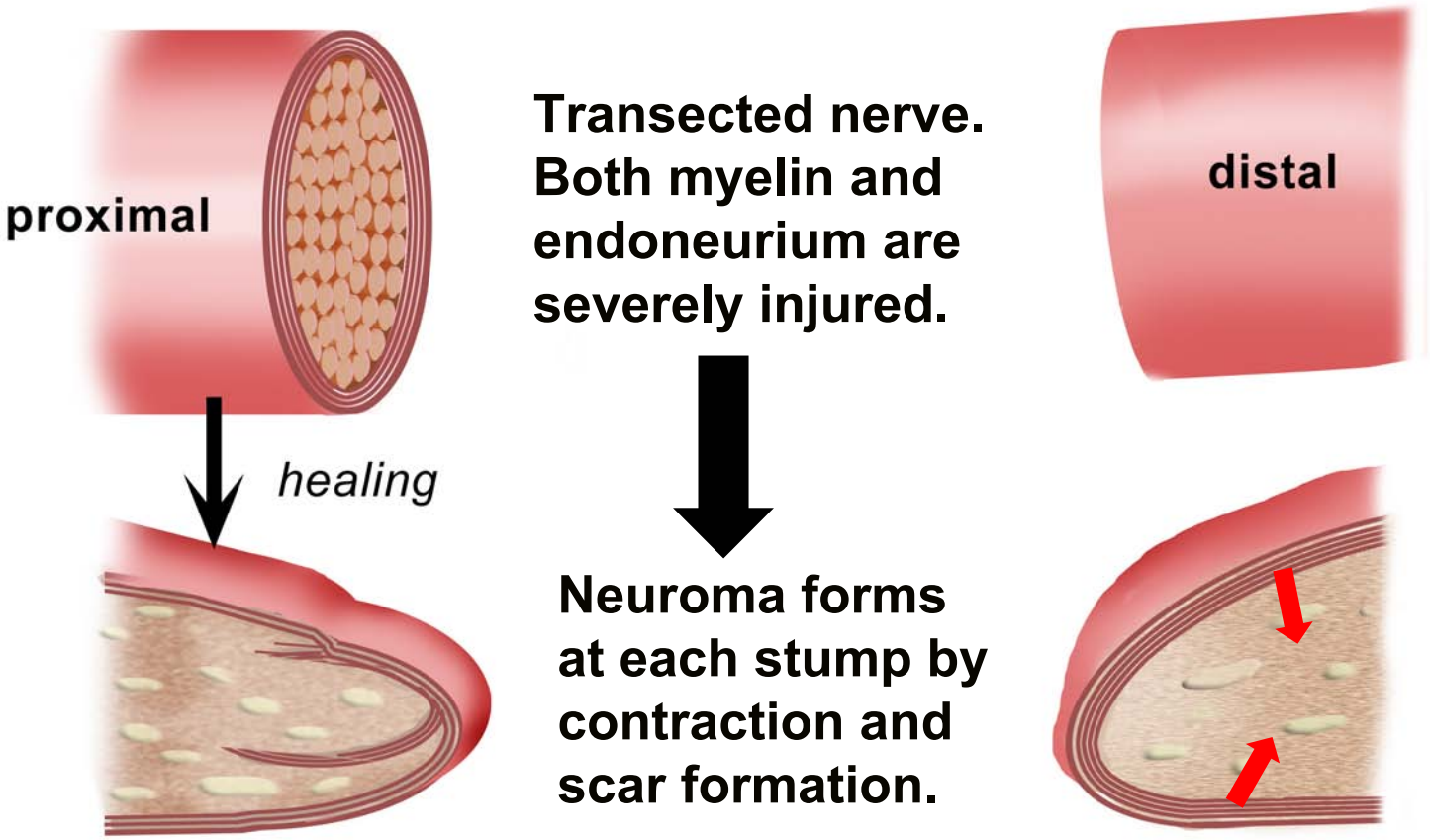


Figure by MIT OCW.

The endoneurial stroma is a nonregenerative tissue. Following transection, it forms neural scar (neuroma). Irreversible injury.
Closes with contraction and scar formation.

Yannas, 2001

Summary:

Increased severity of injury ——————→

	Regenerative tissues. Reversible injury. No contraction.	Nonregenerative tissues. Irreversible injury. Contraction+scar.
SKIN	epidermis	dermis (stroma)
	BM	
NERVE	myelin	endoneurial stroma
	BM	

C. Facts: Antagonistic relation between contraction and regeneration.

- **Methodology: defect closure rule.**
- **Four sets of data showing changes in importance of healing modes (C, S, R) with :**
  1. **Development.**
  2. **Severity of organ injury.**
  3. **Scaffold-induced regeneration in adults.**
  4. **Impairment of healing.**

Quantitative description of healing processes: The defect closure rule.

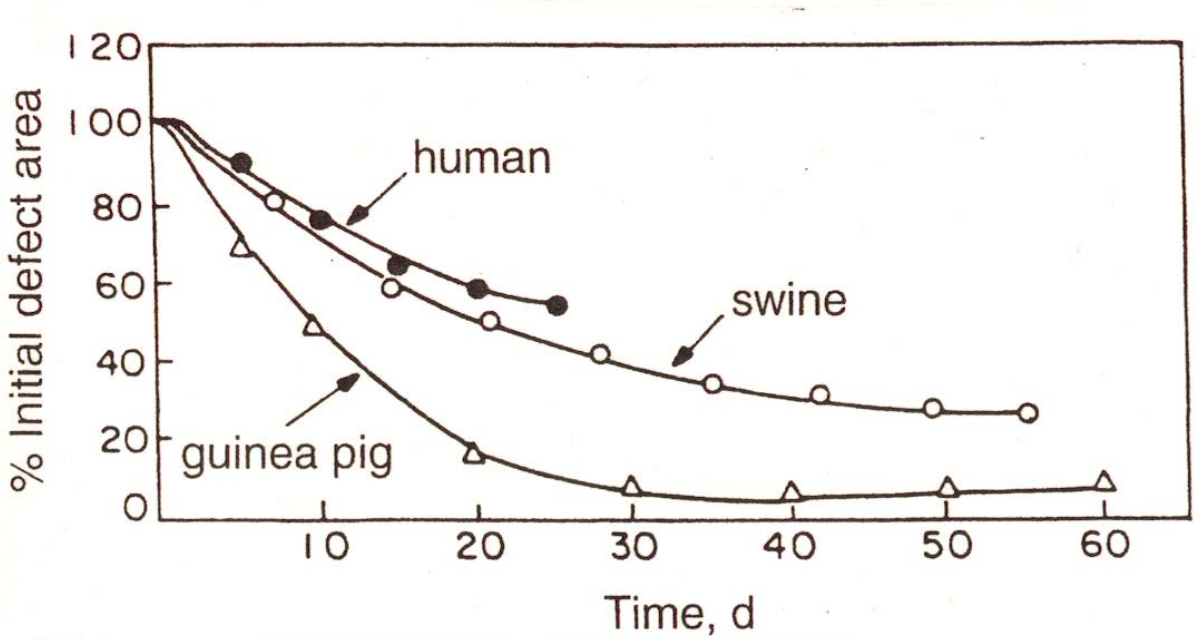
Separate mechanism from final state!

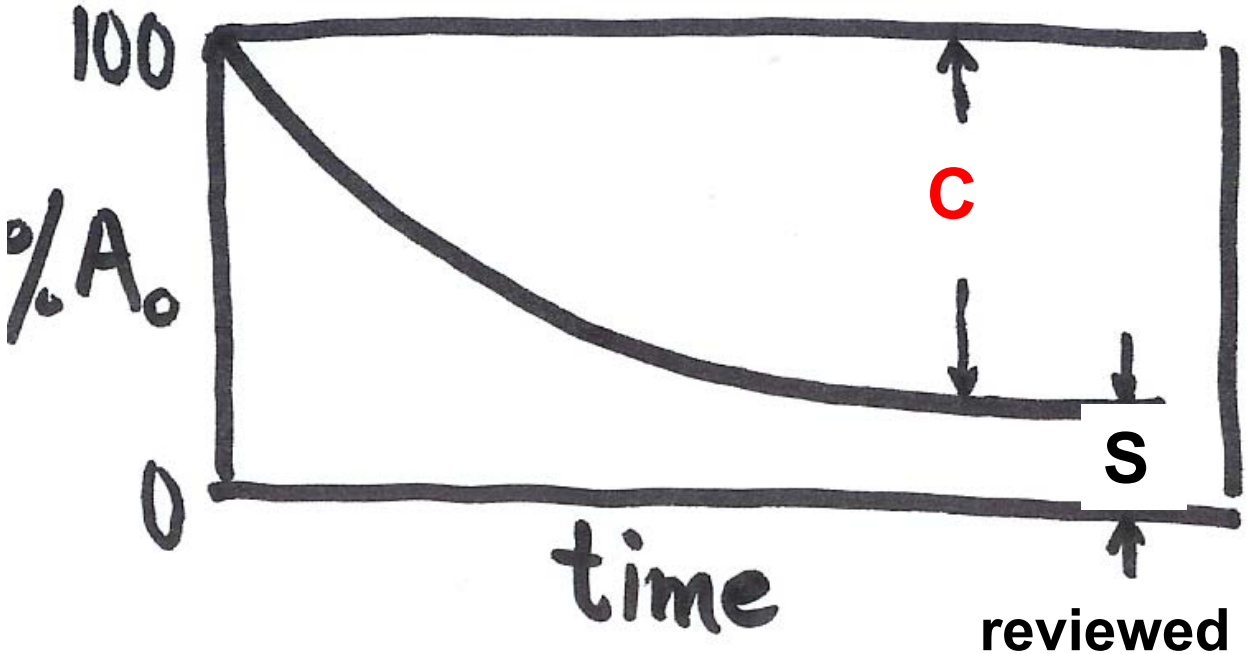
- The initial state is the freshly injured wound. Wound area is A_o.
- The final state is the closed wound. A_o eventually has closed up by three processes: contraction, scar formation, regeneration. No other processes involved in wound closure.
- Closure of wound by contributions from contraction (%C), scar formation (%S) or regeneration (%R).

Defect closure rule:

$$C + S + R = 100$$

Measurement of C, S and R in full-thickness skin wounds after wound has closed. Use only "final state" data!





reviewed in Yannas, 2001

Representative data illustrating the defect closure rule

Spontaneously healing defect	Configuration of final state
general case	[C, S, R]
Ideal fetal healing	[0, 0, 100]
Dermis-free skin/ adult rodents	[96, 4, 0]
Dermis-free skin/ adult human	[37, 63, 0]
Peripheral nerve/ adult rat	[96, 4, 0]
Conjunctiva/ adult rabbit	[45, 55, 0]

Data reviewed in Yannas, 2001

Data set 1: Change in healing modes (C, S, R) with development

- During the fetal-to-adult transition in mammals contraction gradually replaces regeneration as the major mode of wound closure (Lorenz et al., 1992; Mast et al., 1992; Stocum, 1995; McCallion and Ferguson, 1996; Martin, 1997).
- During amphibian development contraction becomes dominant and scar appears as regeneration recedes (Stocum, 1995; Tsonis, 1996; Yannas et al., 1996).

Tadpole development → Frog

Developmental changes in configuration of final state [C, S, R]:

Development ——————————————————→

[41, 0, 59] → [62, 0, 38] → [66, 0, 34] → [90, 10, 0]

tadpole → frog

Yannas et al., 1996

Data set 2: Scaffold-induced regeneration in adults

a. Regeneration is induced when a scaffold blocks contraction. Three organs: Skin, conjunctiva, peripheral nerve.
b. Scar is abolished when contraction is blocked by a scaffold, even modestly.

Comment: At least in rodents, scar formation appears to be a process secondary to contraction.

Data illustrating use of active scaffolds in 3 organs

Organ/ species	Treatment used	Spontaneous healing	Treated with template
Skin/guinea pig	scaffold DRT	[91, 9, 0]	[89, 0, 11]
Skin/guinea pig	scaffold DRT+ KC	[92, 8, 0]	[28, 0, 72]
Conjunctiva/ rabbit	scaffold DRT	[45, 55, 0]	[13, 0, 87]
Nerve/rat	silicone tube+scaffold NRT	[95, 5, 0]	[53, 0, 47]
Nerve/rat	collagen tube+scaffold NRT	[95, 5, 0]	[0, 0, 100]

Data reviewed in Yannas, 2001

Kinetics of closure of skin defect area using three protocols

KC = keratinocytes
DRT = dermis regeneration template (active scaffold)

Graph of % initial defect area vs. time - removed for copyright reasons.

adapted from Yannas et al., 1989

Myofibroblast detected with antibody to α-SM actin

Diagram removed for
copyright reasons.

Tomasek et al., 2000

Contraction blocked by scaffold (bottom)

Ungrafted.
<u>Contracting vigorously</u>.

→

Photo removed for copyright reasons.

Red-brown: stained with antibody to α-SM actin. 10 d

Grafted with DRT.
<u>No contraction</u>.

→

Photo removed for copyright reasons.

Troxel, 1994

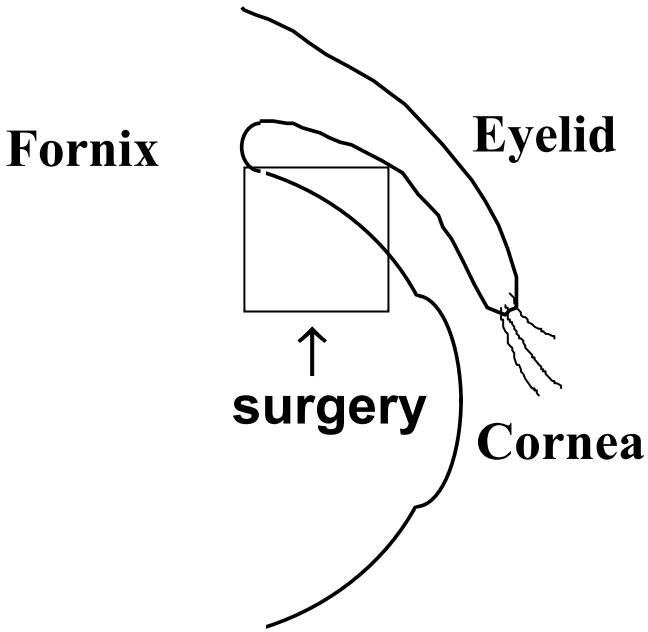
Mechanism of contraction inhibition by DRT scaffold in skin wound

1. Fact: Reduction in number of myofibroblasts.

2. Fact: Disruption of myofibroblast organization.

Injured conjunctiva model

(excise full-thickness conjunctiva including entire stroma, then graft with scaffold)



Low mag (eyeball)

DRT = dermis regeneration template in proximal layer (grafted on wound)

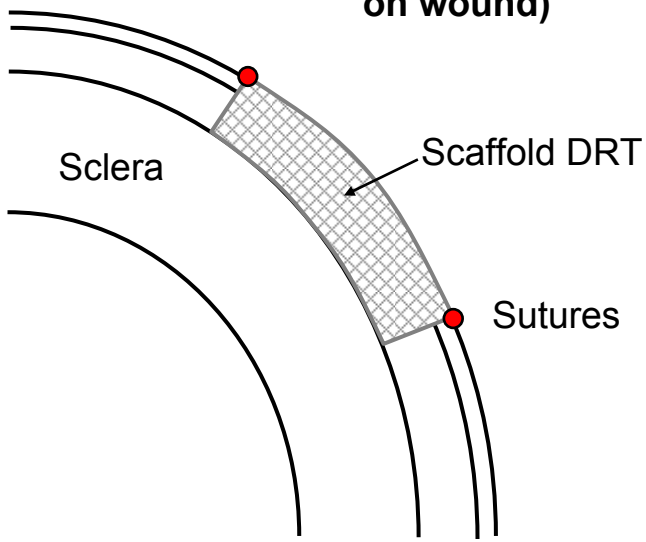
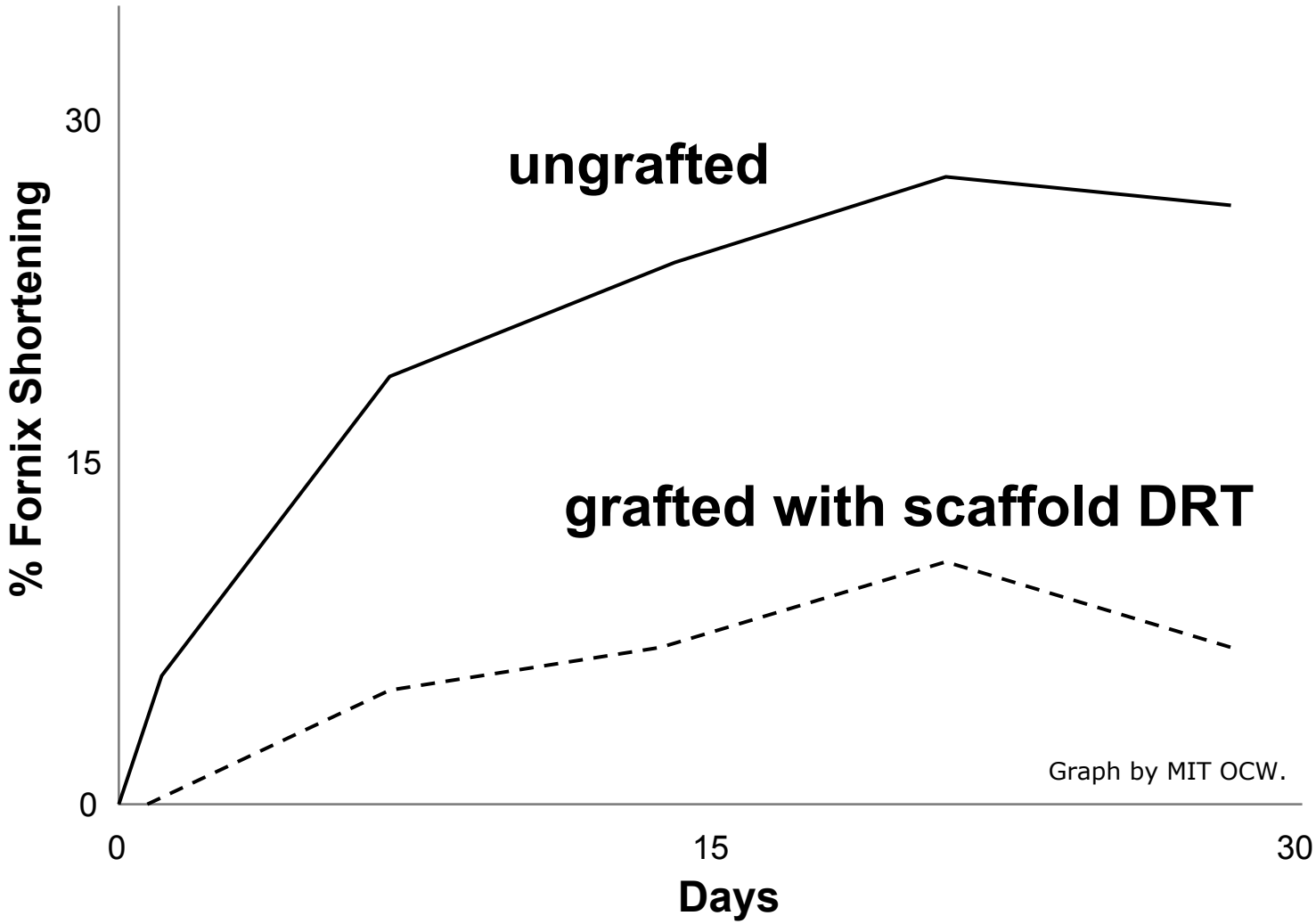


Figure by MIT OCW.

High mag (grafted conjunctiva)

DRT graft blocked contraction of conjunctival wound



Hsu et al., 2000

Data set 3. Impaired healing of skin wounds

Dermis-free wounds in:

- **genetically diabetic mouse**
- **genetically obese mouse**
- **infected wounds**
- **mechanically splinted**
- **treated with steroids**

all impaired-healing wounds showed strong delay in contraction but did not show regeneration

Data from: Lindquist, 1946; Billingham and Russell, 1952; Cuthbertson, 1959; Abercrombie et al., 1960; Zahir, 1964; Stone and Madden, 1975; Kennedy and Cliff, 1979; McGrath, 1982; Klingbeil et al., 1991; Greenhalgh et al., 1990; Fiddes et al., 1991; Hayward et al., 1992.

Summary of Data Sets 1-3.

1. During amphibian larval (tadpole) development;
   also, during the fetal-to-adult transition in mammals:
   C↑ R↓
2. Certain scaffolds block contraction and induce partial regeneration in adult mammals (rodents, swine, human).
   C↓ R↑
   Also scar is abolished when contraction is blocked, even partly.
   C↓ S = 0
3. Impaired healing blocks contraction but does not induce regeneration.
   C = 0 R = 0

How does an active scaffold block contraction? Identify structural determinants of scaffold activity.

Critical structural features of biologically ECM analogs used as scaffolds

1. chemical composition (ligand identity)

2. pore structure (ligand density)

3. orientation of pore channels (ligand spatial coordinates)

4. macromolecular structure (scaffold duration)

Diagram removed for copyright reasons.

The graphic shows many scaffolds but dermis regeneration template (DRT) is the active scaffold (template). Ligand density is optimal between 20 and 120 μm,

Graph removed for
copyright reasons.

Yannas et al., 1989

Structural determinants of regeneration template activity

Structural parameter of scaffold	Scaffold induces SKIN regeneration*	Scaffold induces NERVE regeneration**	Contribution to regenerative activity
Type I collagen/GAG, w/w	98/2	98/2	Ligand identity → Myofibroblasts (MFB) bound on scaffold
Average pore diameter, μm	20-120	5-10	Ligand density → Almost all MFB bound on scaffold
Pore channel orientation	random	axial	Spatial coordinates of ligands → Morphology of new organ
Average molecular weight between crosslinks****, M_c, kDa	5-15	40-60	Duration of scaffold topology → Synchronization with synthetic process
Degree of residual collagen fiber crystallinity (residual banding)***	ca. 5% of native collagen	ca. 5% of native collagen	Inhibition of platelet-aggregation → Reduce number of myofibroblasts

D. Facts: Isomorphous replacement

Must explain not only contraction blocking but also synthesis of organ

Rules of Organ Synthesis

Rule 1. Isomorphous Replacement

Stroma regeneration proceeds on the surface of a matrix that is a replica of the native stroma of the organ.

Rule 2. Synchronous Tissue Synthesis

The template is required to remain intact (undegraded) long enough to initiate synthesis of new stroma but not long enough to block sterically the synthesis of new tissues.

Summary of stroma synthesis. A scaffold cannot induce organ synthesis unless it is a configurational replica of the desired stroma and unless it degrades at a rate equal to the rate of stroma synthesis at the injured anatomical site.

E. Theories of regeneration.

1. Contraction blocking and isomorphous replacement.

2. Immunocompetence theory.

Contraction blockade theory explains Data Sets 1-3

symbols refer to [C, S, R]

- **Inhibition of contraction is necessary but does not suffice to induce organ regeneration in adults**

$$\Delta R > 0 \text{ and } S \rightarrow 0 \text{ if } \Delta C < 0$$

Explain facts of regeneration using unified theory:

Contraction blockade +

+ Isomorphous replacement $\longrightarrow$

$\longrightarrow$ Regeneration

Alternative theories of induced organ regeneration in adults

1. Increase in immune competence during development controls the gradual loss of regenerative potential that accompanies metamorphosis in amphibians and the fetal-adult healing transition in adults (Heber-Katz, 1999; Harty et al., 2003).
2. Regeneration is induced in adults by a scaffold that blocks contraction and provides a topology similar to the stroma being regenerated, remaining intact only for the duration of organ synthesis (Yannas, 2001).

Two theories of transition in healing response

1. Fetal → immune competence development → Adult

2. Adult → template → Fetal