Macroscopic forces generated by cell-matrix interactions

I. Cells generate forces after becoming attached to a matrix.

II. How do cells attach to a matrix?

III. Cell-matrix interactions control the spontaneous closure of wounds in organs.

IV. What happens when wound closure occurs by induced regeneration?

Outline

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

B. Irreversible organ injury.

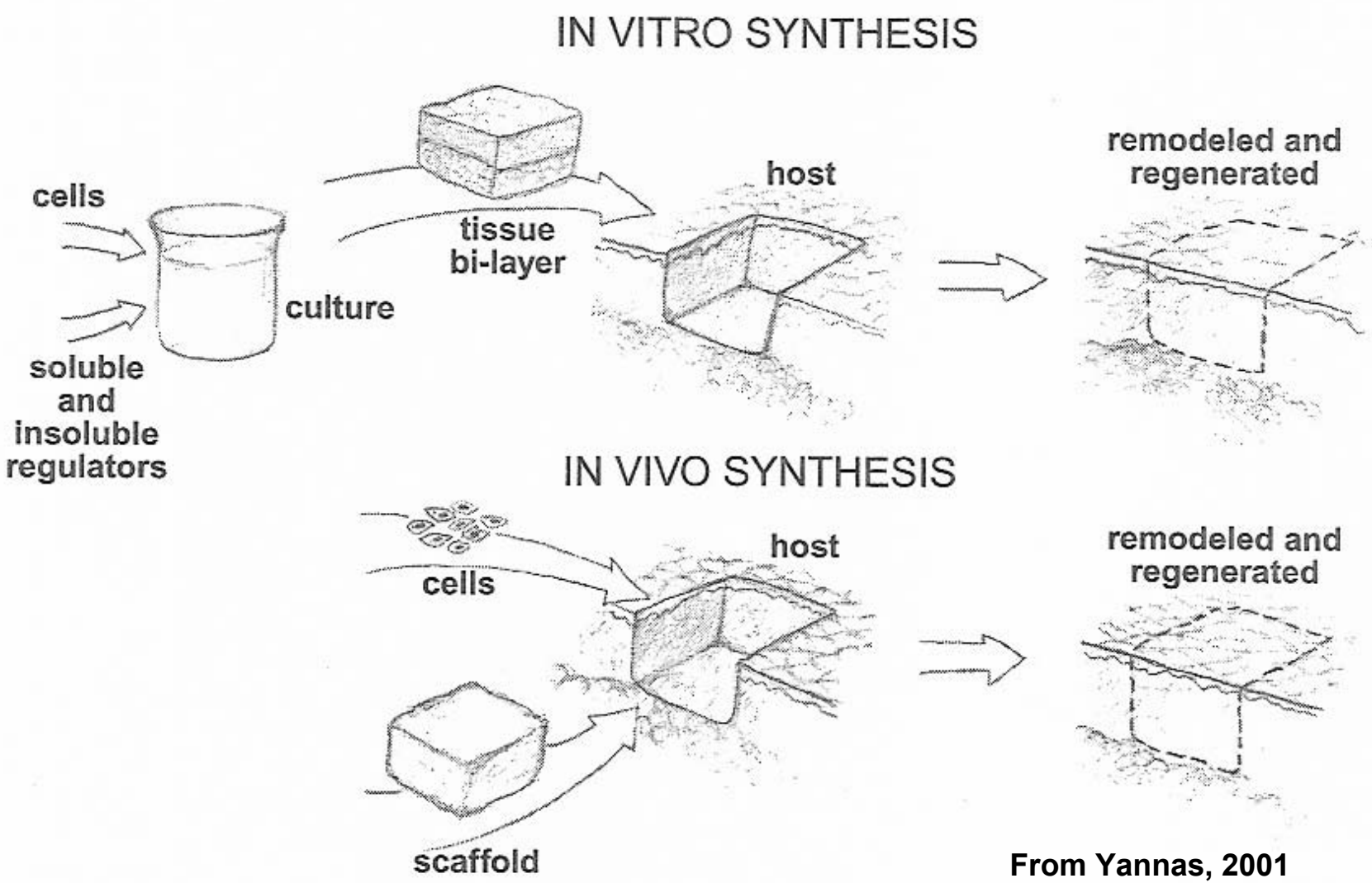
C. Antagonistic relation between contraction and regeneration.

D. Synchronous and isomorphous replacement.

E. Two theories. 1. Immunocompetence theory. 2. Contraction blockade + synchronized isomorphous replacement.

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

Skin: In vitro or in vivo synthesis?



From Yannas, 2001

Peripheral nerves: In vitro or in vivo synthesis?

Diagram removed for copyright reasons.
See Figure 7.1 in [Yannas 2001]: Yannas, I. V. *Tissue and Organ Regeneration in Adults*. New York: Springer, 2001.

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 2001].

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

C. 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 :**

I. **Development.**
II. **Severity of organ injury.**
III. **Scaffold-induced regeneration in adults.**
IV. **Impairment of healing.**

Quantitative description of healing processes: The defect closure rule.

!separate mechanism from final state!

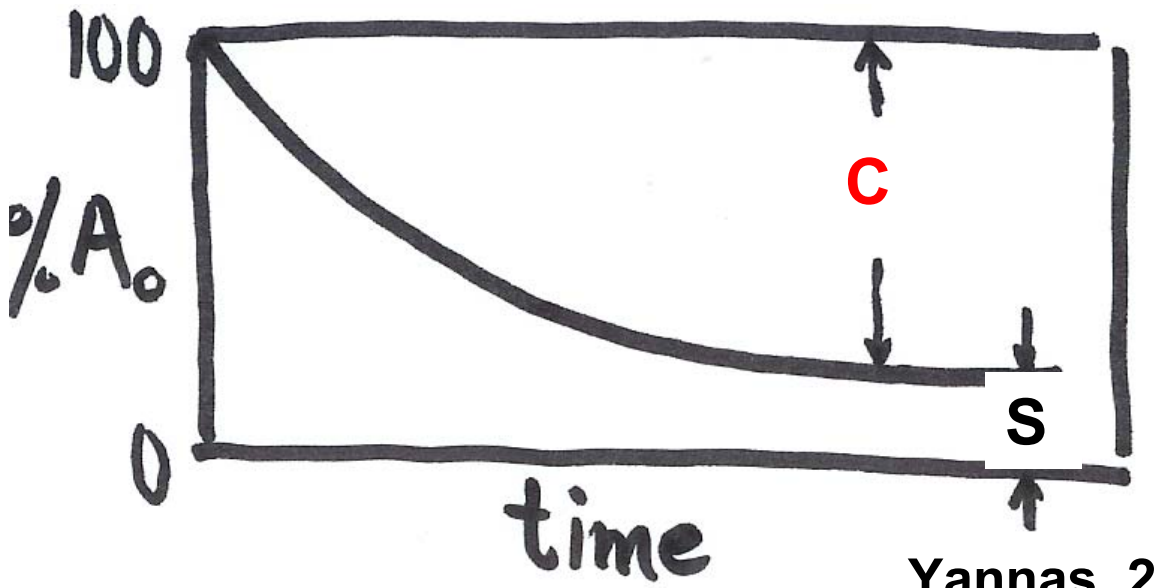
- The initial state is the freshly injured wound. Wound area is A_o.
- The final state is the closed wound. A_o eventually has closed up by three processes: contraction, scar formation, regeneration. No other processes involved in wound closure.
- Closure of wound by contributions from contraction (%C),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

Graph removed for copyright reasons.
See Figure 4.3 in [Yannas 2001].



Yannas, 2001

Burn patient has closed severe skin wounds in neck partly by contraction and partly by scar

Photo removed for copyright reasons.

Final state of healing of full-thickness skin wound in the human.

Final state of healing of full-thickness skin wound in the guinea pig.

Orgill, *MIT Thesis*, 1983

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

Fact I: 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

Tadpole development ——————→ | Frog

Graph removed for copyright reasons.
Percent wound closure across developmental stages.
See Figure 8.3 in [Yannas 2001].

Fact II: Changes in healing modes (C, S, R) with severity of organ injury

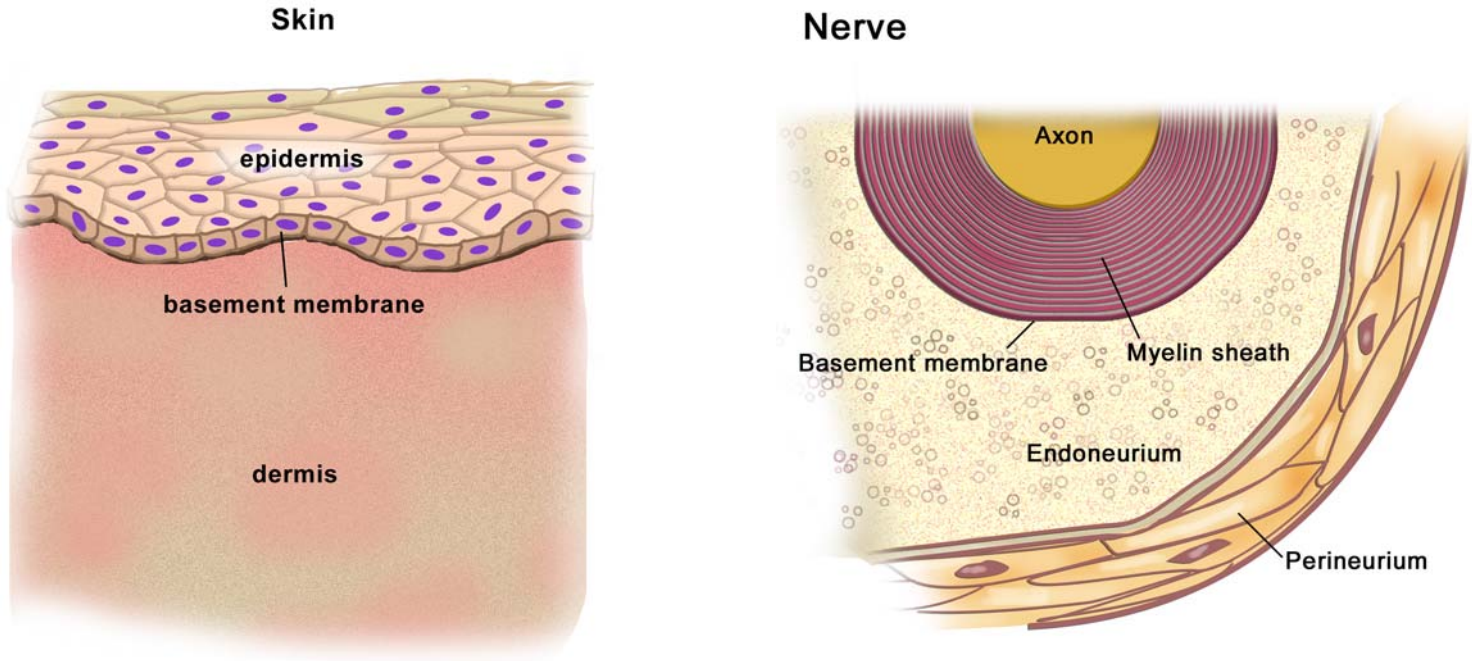
- As injury progresses in severity in an organ from epithelial tissue to basement membrane to stroma, contraction replaces regeneration as the major mode of wound closure .

The tissue triad in skin and nerves

epithelial tissue: 100% cellular, no ECM

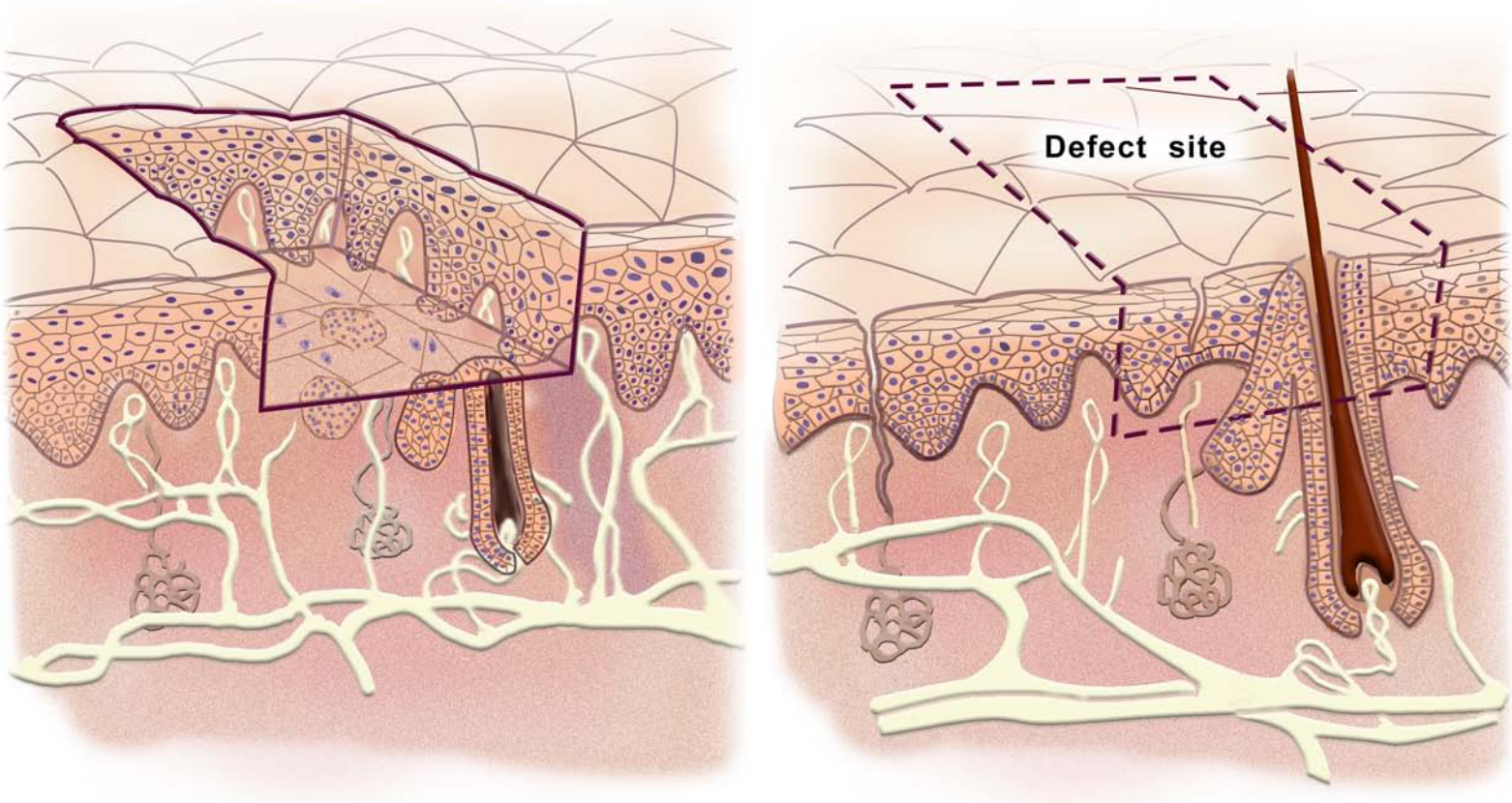
basement membrane: 100% ECM , no cells

stroma: cells, ECM, blood vessels



Figures by MIT OCW. After Figure 2.7 in [Yannas 2001].

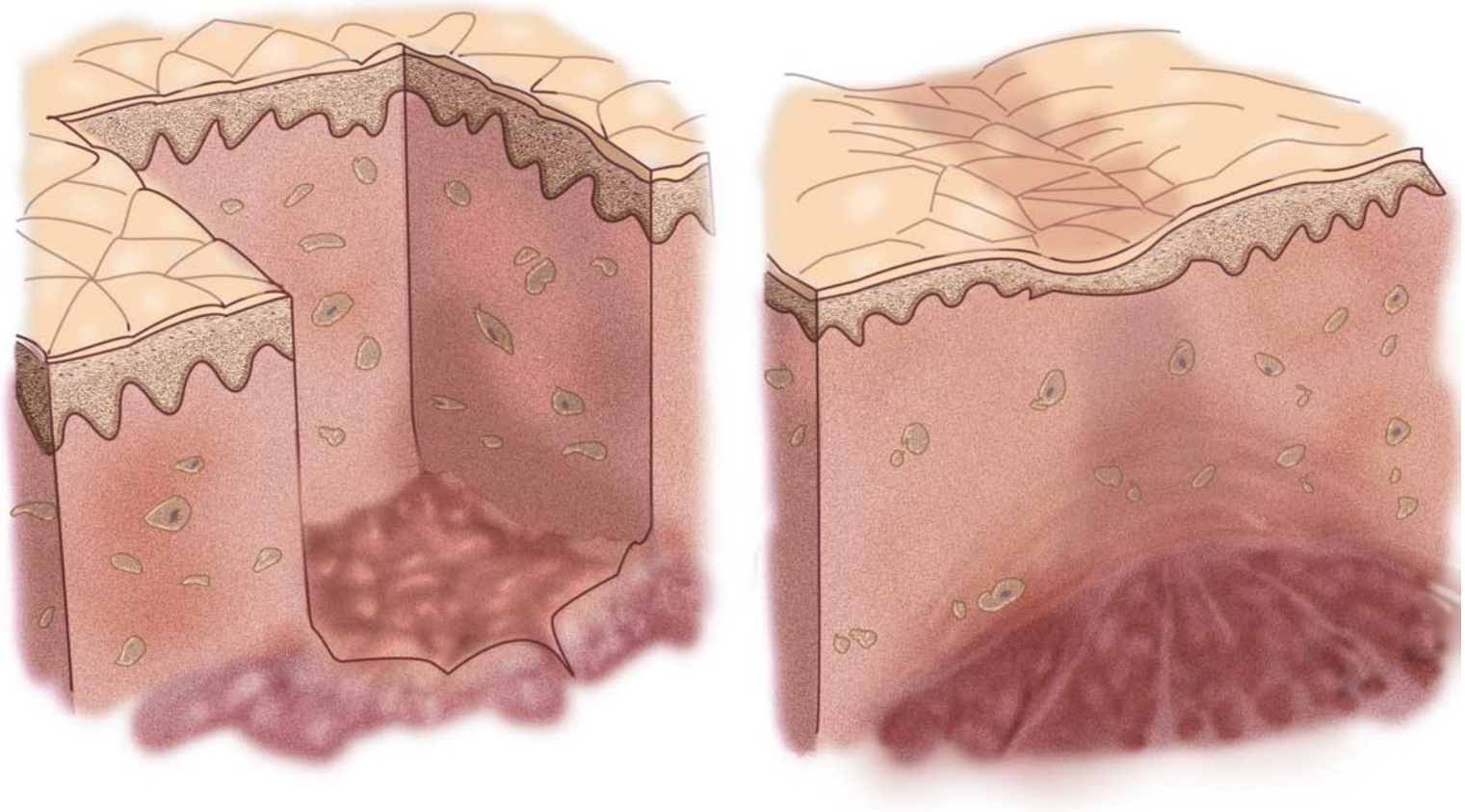
Skin: reversible injury



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

Figure by MIT OCW. After Figure 2.1 in [Yannas 2001].

Skin: Irreversible injury

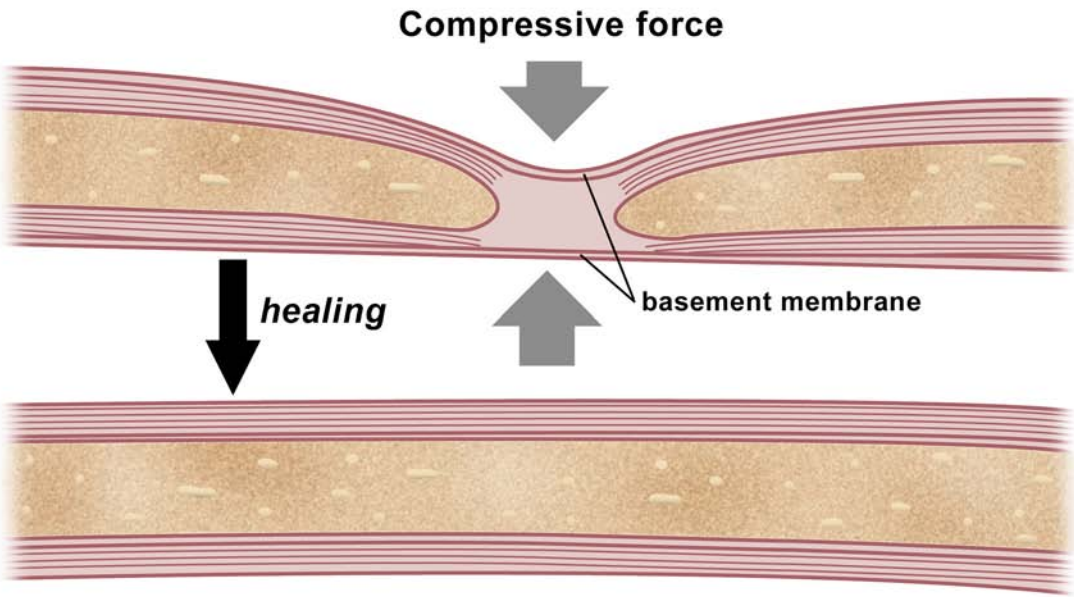


The dermis is a nonregenerative tissue in the adult. After excision, it does not regenerate spontaneously. Irreversible injury. Closes with contraction and scar formation.

Figure by MIT OCW. After Figure 2.2 in [Yannas 2001].

Peripheral nerve: reversible injury

crushed nerve heals spontaneously by regeneration

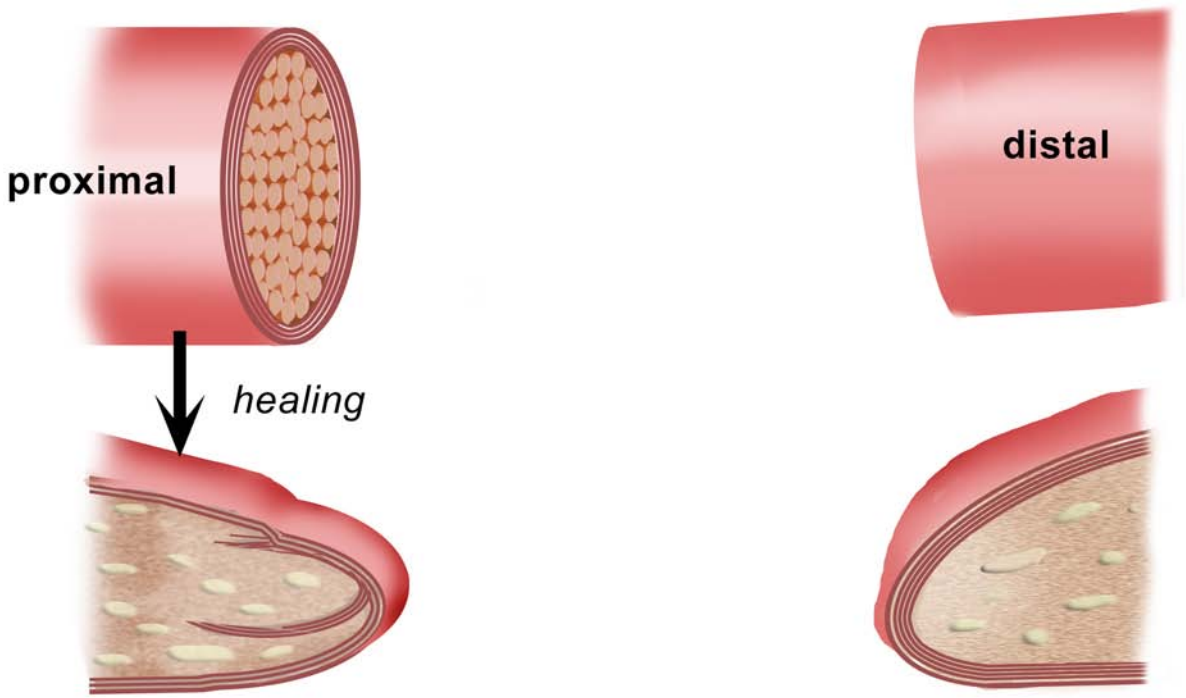


The myelin sheath is a regenerative tissue. Following nerve crushing with myelin disruption, the myelin regenerates spontaneously. Reversible injury. No contraction. No scar.

Figure by MIT OCW. After Figure 2.3 in [Yannas 2001].

Peripheral nerve: irreversible injury

transected nerve heals spontaneously by contraction and neuroma (neural scar) formation



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

Figure by MIT OCW. After Figure 2.4 in [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	

Fact III: 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.

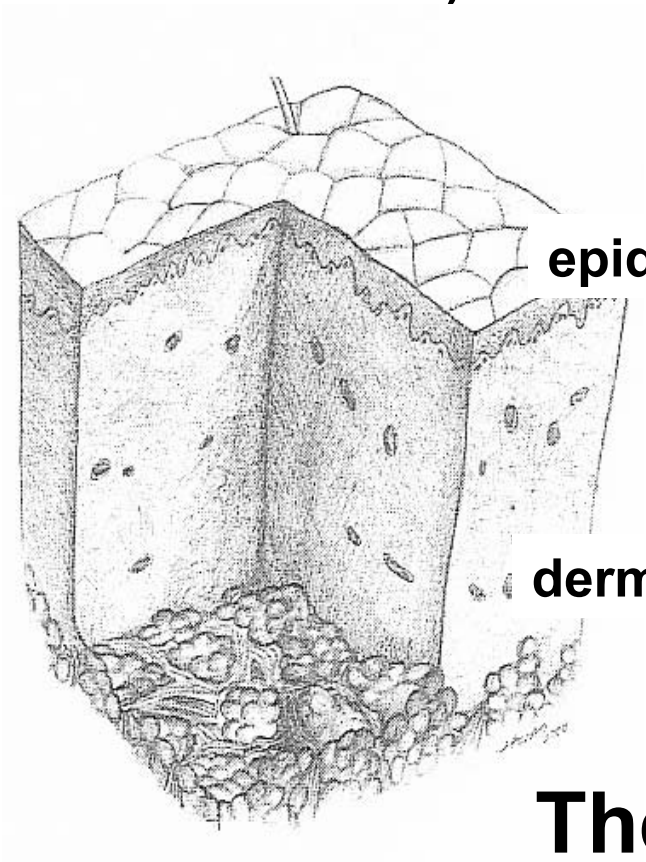
Data illustrating use of active scaffolds in 3 organs

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

Standard injured skin model

(excise full-thickness skin, including entire dermis, then graft with scaffold)



Yannas, 2001

The initial state of skin wound healing studies

Kinetics of closure of skin defect area using three protocols

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

Graph removed for copyright reasons.
See Figure 10.2 in [Yannas 2001].

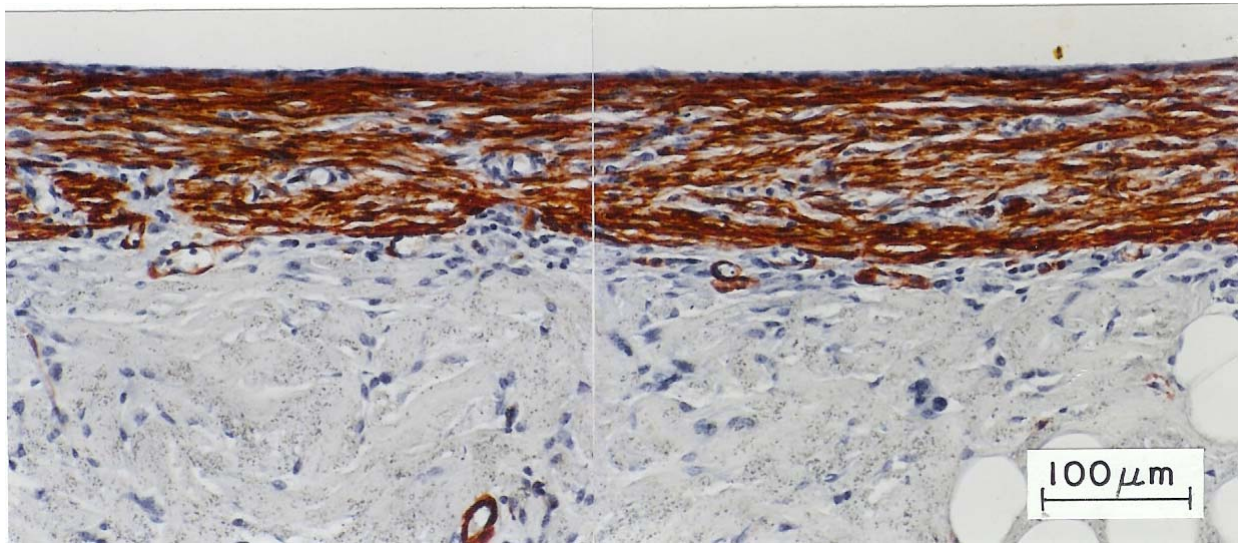
Myofibroblast detected with antibody to α-SM actin

Diagram removed for copyright reasons.

Tomasek et al., 2000

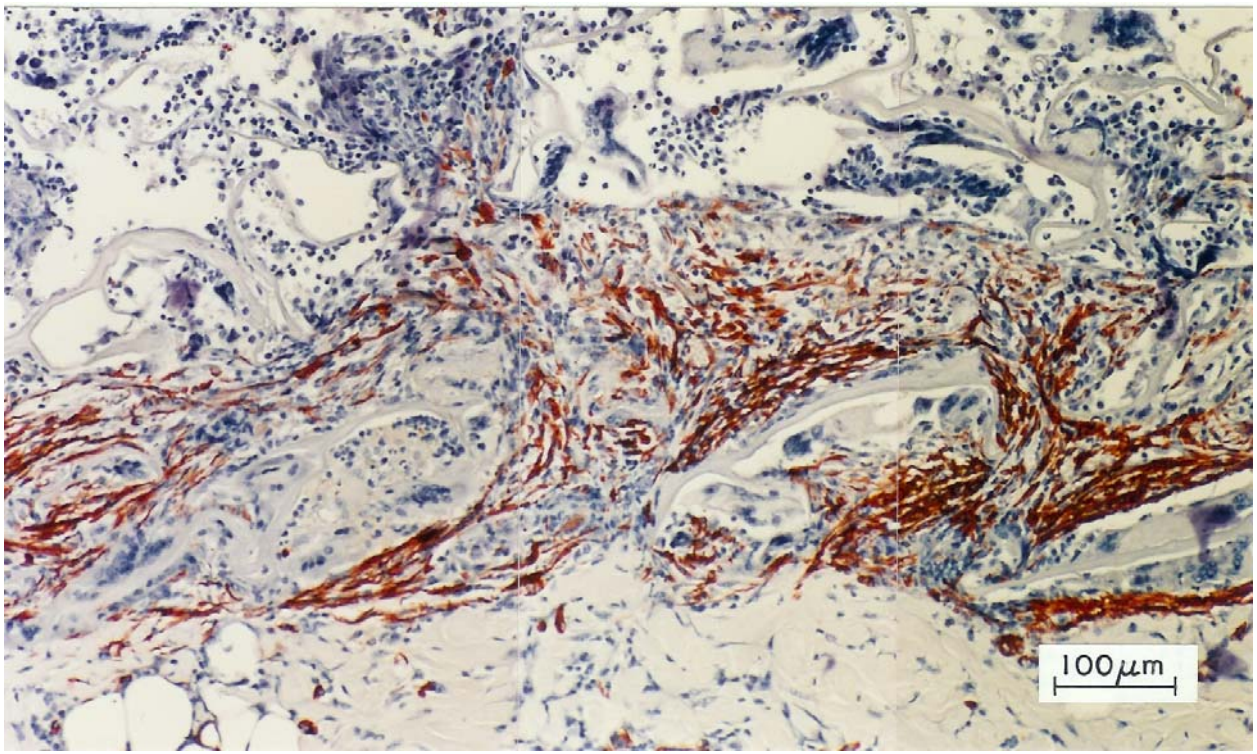
Contraction blocked by scaffold (bottom)

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

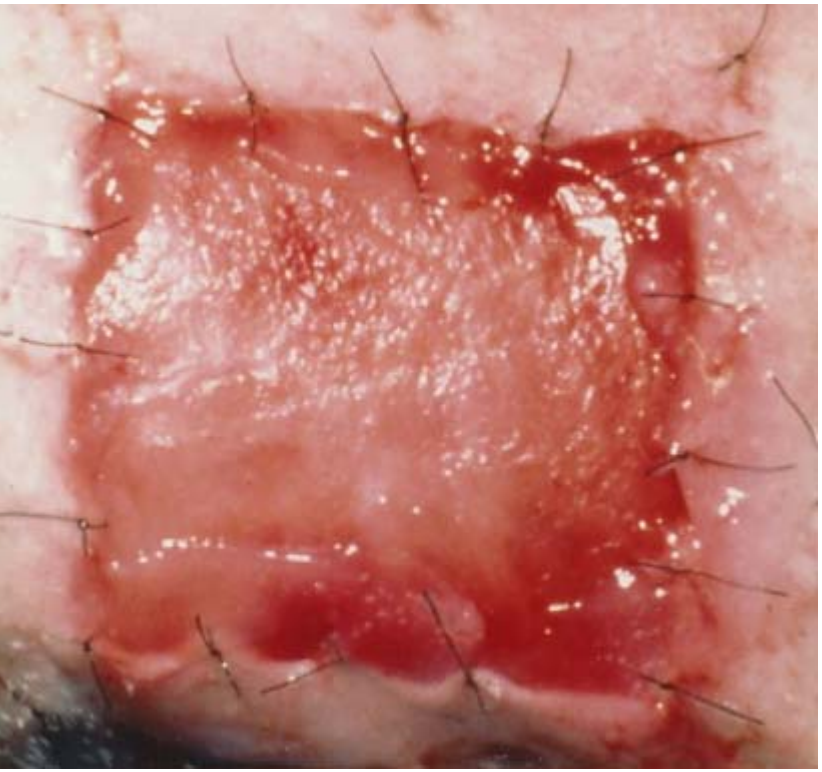


Red-brown: stained with antibody to α-SM actin. 10 d

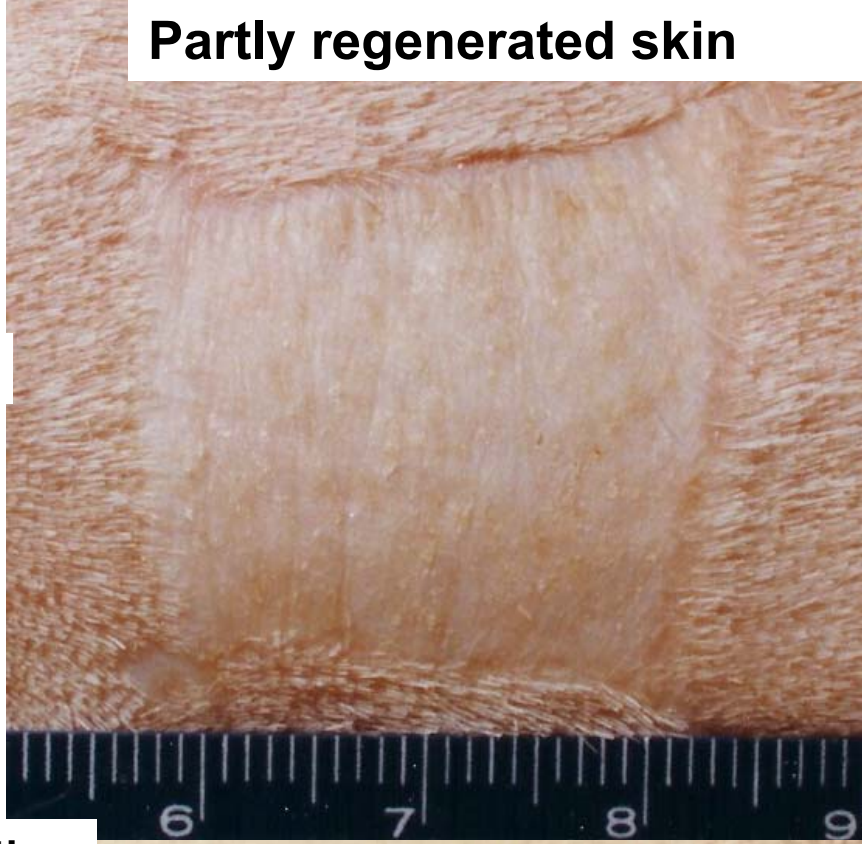
Grafted with DRT. <u>No contraction</u>.



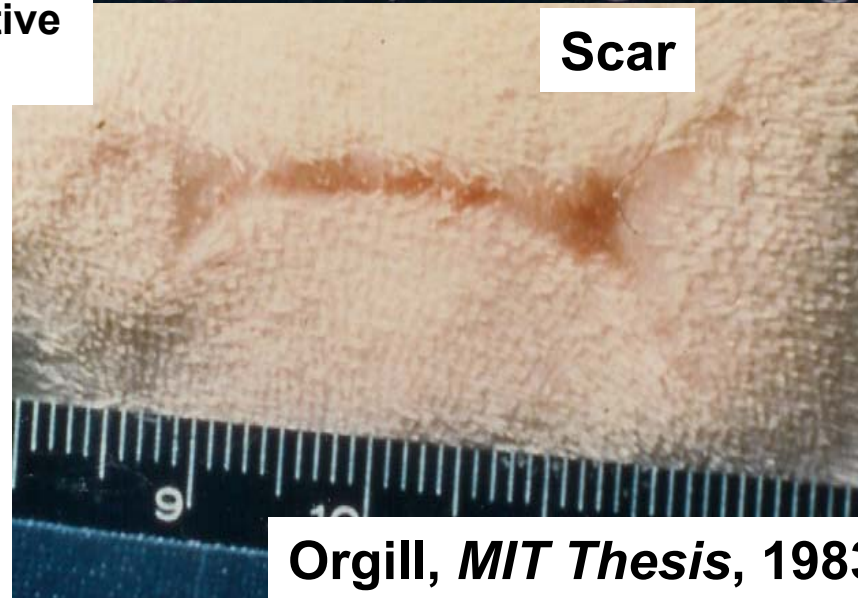
Troxel, *MIT Thesis*, 1994



KC + DRT



KC + inactive scaffold



Full-thickness skin wound (guinea pig) grafted with keratinocytes (KC) and either dermis regeneration template (DRT) or inactive scaffold

Orgill, *MIT Thesis*, 198

KINETICS OF SKIN SYNTHESIS

E, neoepidermis
D, neodermis

Photos removed for copyright reasons.

Scaffold has partly degraded by 14 d

Butler et al., 1998

Normal skin

rete ridges with capillary loops and vascular plexus underneath

Diagram removed for copyright reasons.
See Figure 5.2 (top left) in [Yannas 2001].

Burkitt et al., 1992

Regenerated skin (swine).
Immunostaining with Factor VIII for capillary loops.
No angiogenesis factors were added by investigators.

Photo removed for copyright reasons.

capillary loops

75 μm

Staining for capillaries forming in loops as part of the rete ridge structure

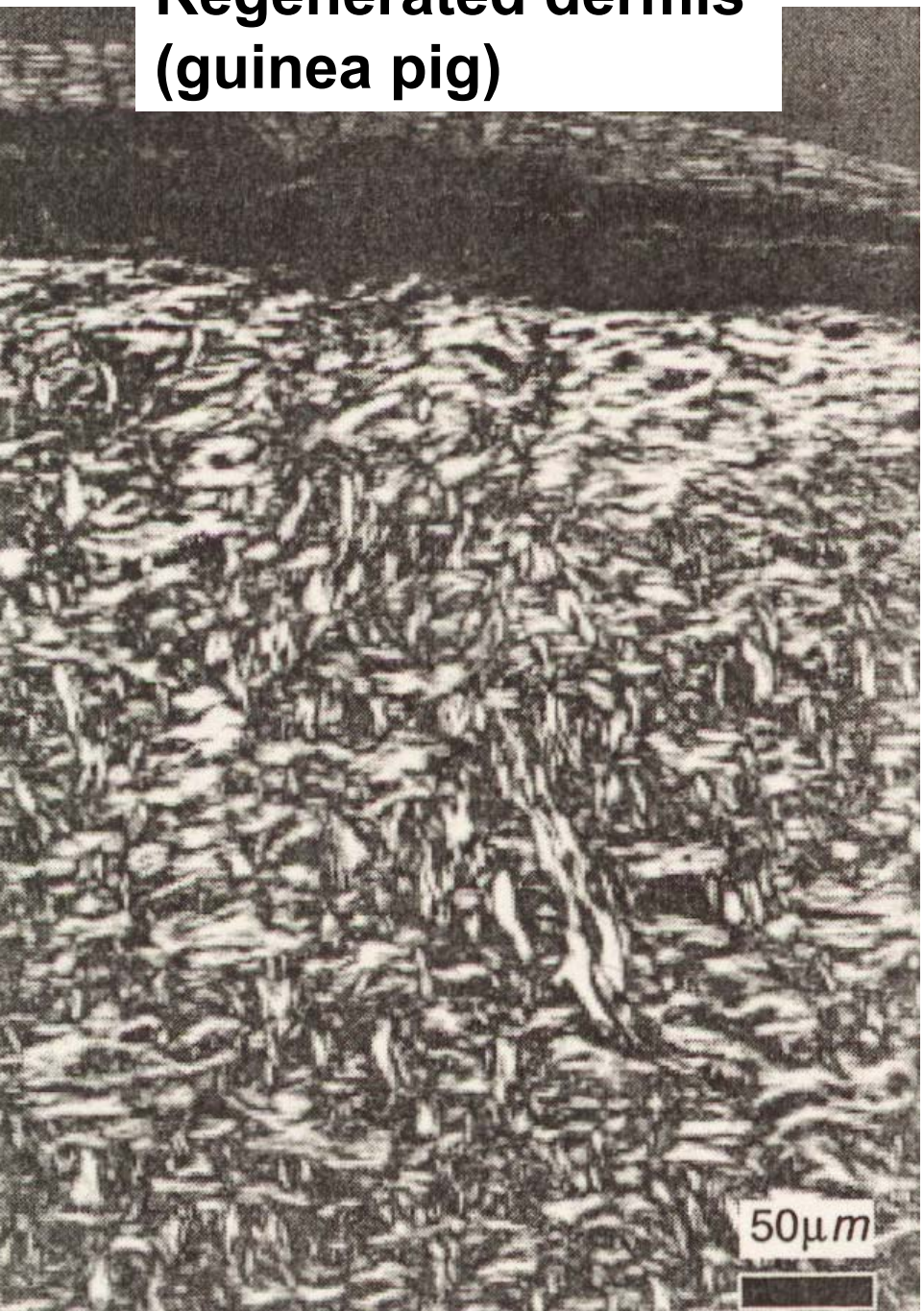
This is our regenerated tissue, closely approximating the normal

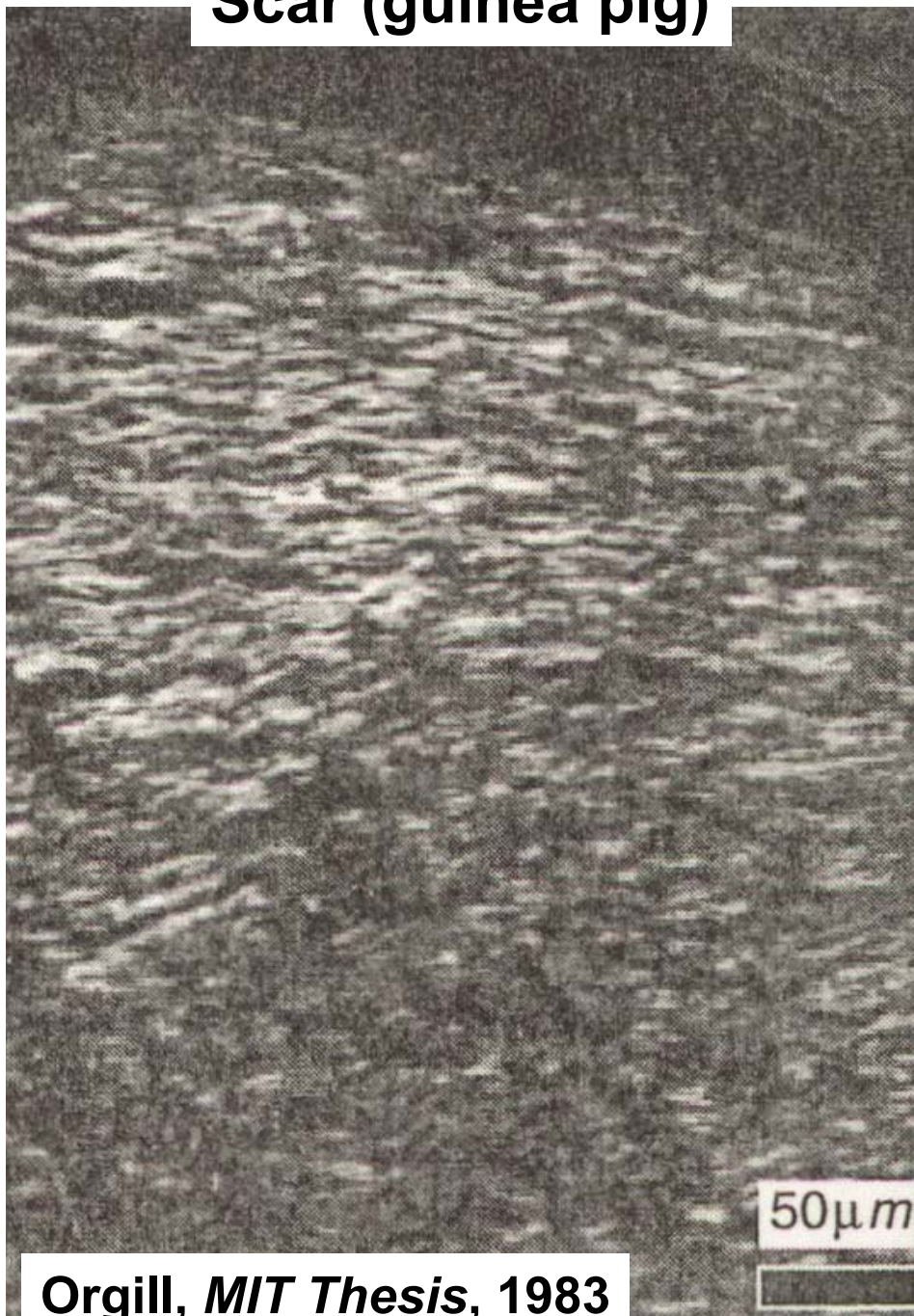
All this tissue was ECM seeded with 50,000 keratinocytes per cm2, porcine model (next three slides are histology following grafting of a 4x4 cm square wound cut down to the fat layer

Day 35:

Vascular remodeling is evident on stains for endothelium that reveal a well organized arcade of hairpin-loop capillaries interdigitating with nascent epidermal rete ridges in a normal pattern

Compton et al., 2000





Orgill, *MIT Thesis*, 1983

Severely burned patient lost all skin on right side of face.
Grafted with DRT scaffold and later covered with autoepidermal graft.

← **no eyebrows**

Photo removed for copyright reasons.

↑

regenerated skin
no beard

Photo from Dr. John Burke

Injured conjunctiva model

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

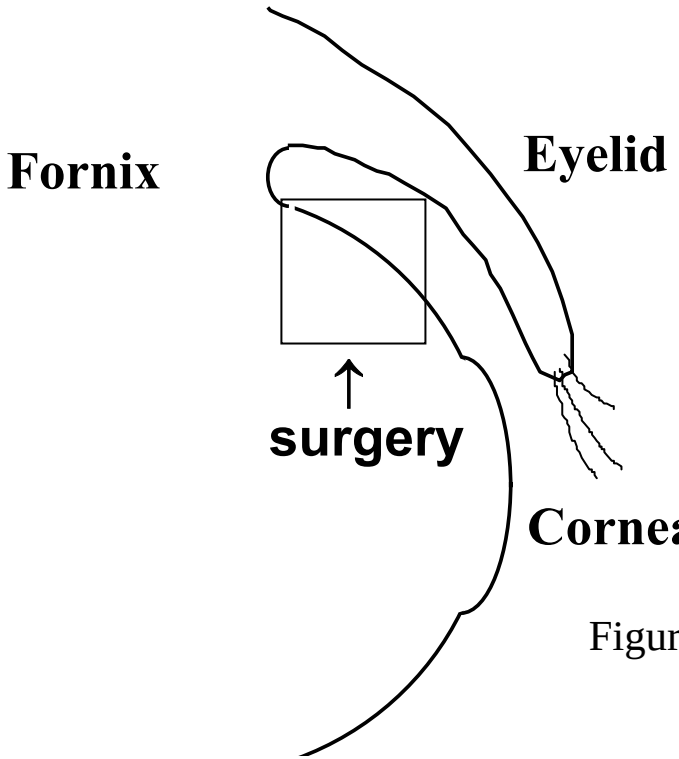
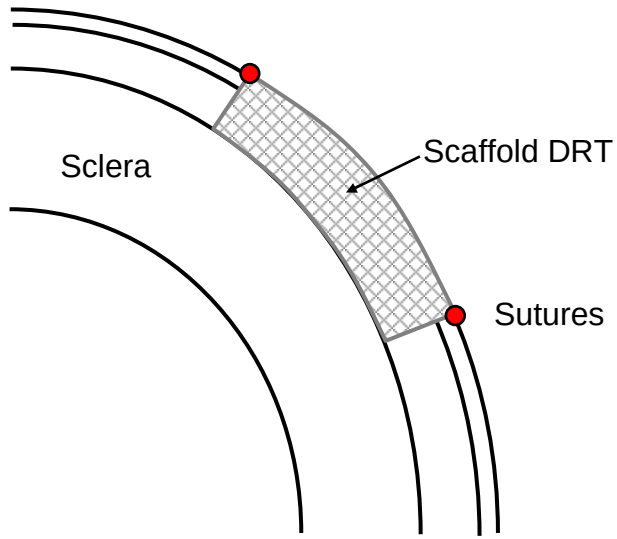


Figure by MIT OCW.



Low mag (eyeball)

High mag (grafted conjunctiva)

After Hsu et al., 2000

DRT graft blocked contraction of conjunctival wound

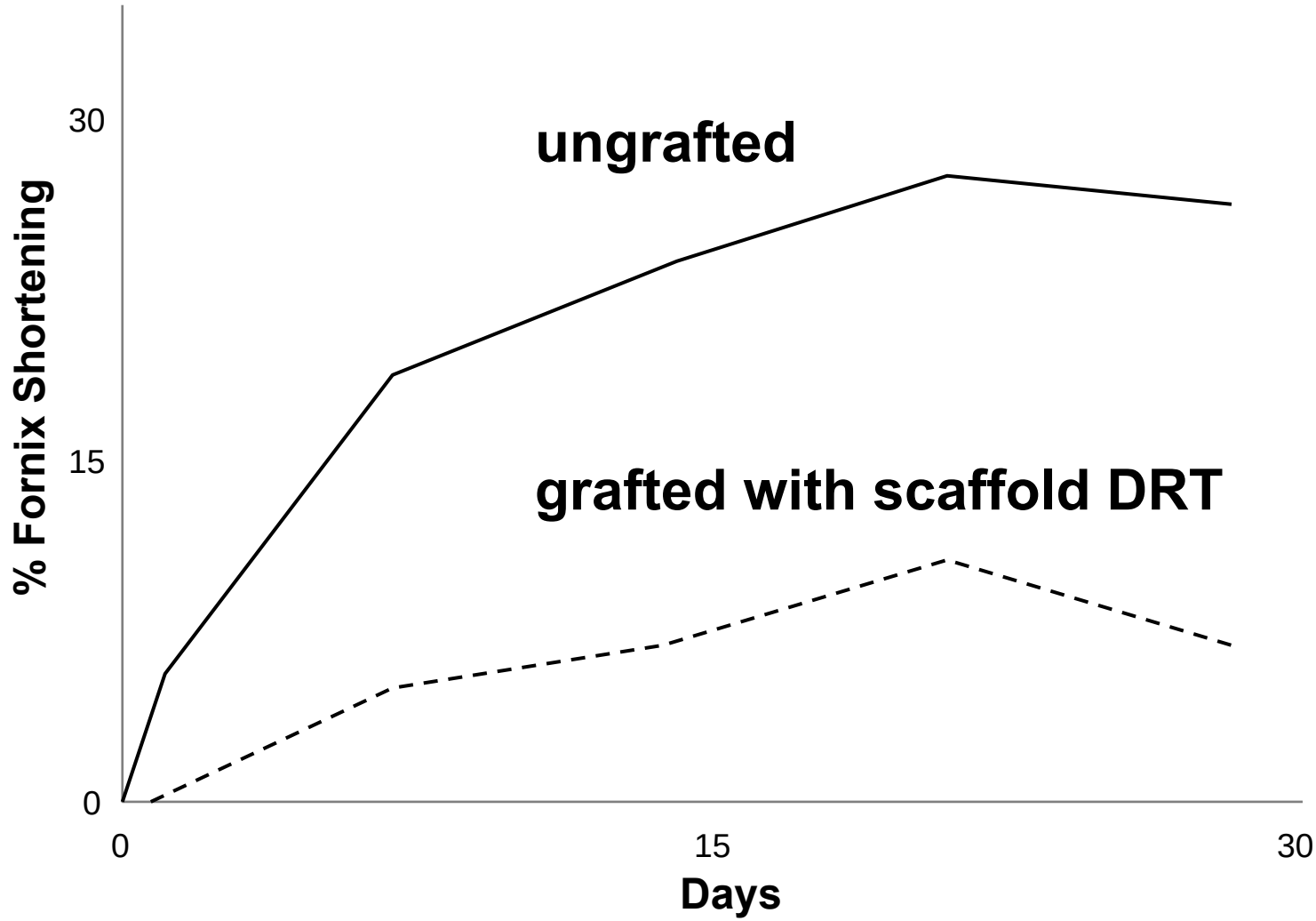


Figure by MIT OCW.

After Hsu et al., 2000

Ungrafted. Contracting vigorously. →

Photo removed for copyright reasons.

Red-brown: stained with antibody to α-SM actin. 14 d

Arrows: newly formed blood vessels

Grafted with DRT. Blocked contraction. →

Photo removed for copyright reasons.

Hsu et al., 2000

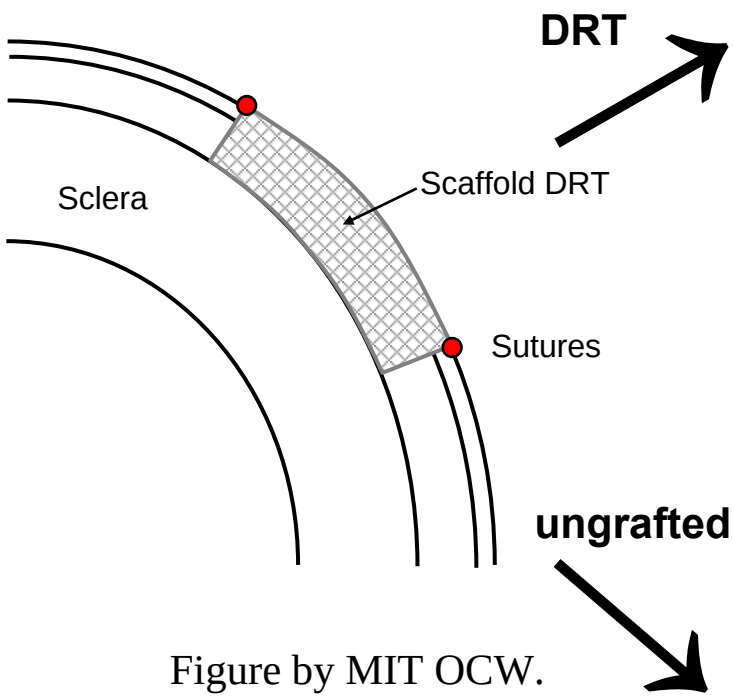


Figure by MIT OCW.

Photo removed for copyright reasons.

regenerated conjunctiva

Photo removed for copyright reasons.

Fully excised conjunctiva (rabbit) grafted with DRT or ungrafted (*14 d*)

scar

Hsu et al., 2000

Regeneration of conjunctival stroma (use microscope polarizing stage to study orientation of collagen fibers)

Photo removed for copyright reasons.

ungrafted (conjunctival scar)

Photo removed for copyright reasons.

grafted with DRT scaffold

Photo removed for copyright reasons.

normal conjunctiva

Photo removed for copyright reasons.

Summary: alignment as indicated by birefringence when viewed under polarized light

These images are histological sections viewed under polarized light. Upper left, ungrafted wound.

Hsu et al., 2000

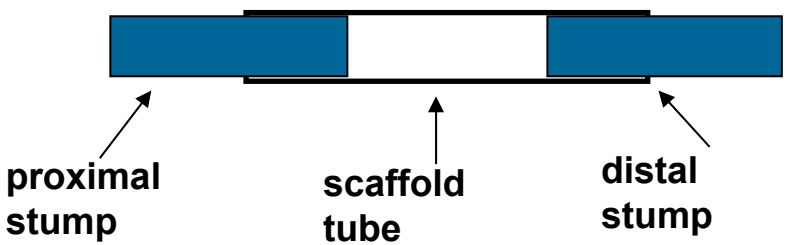
Standard injured peripheral nerve model

(transect nerve, then insert stumps inside scaffold tube)

Diagram removed for copyright reasons.

Cooper & Schiller, 1975

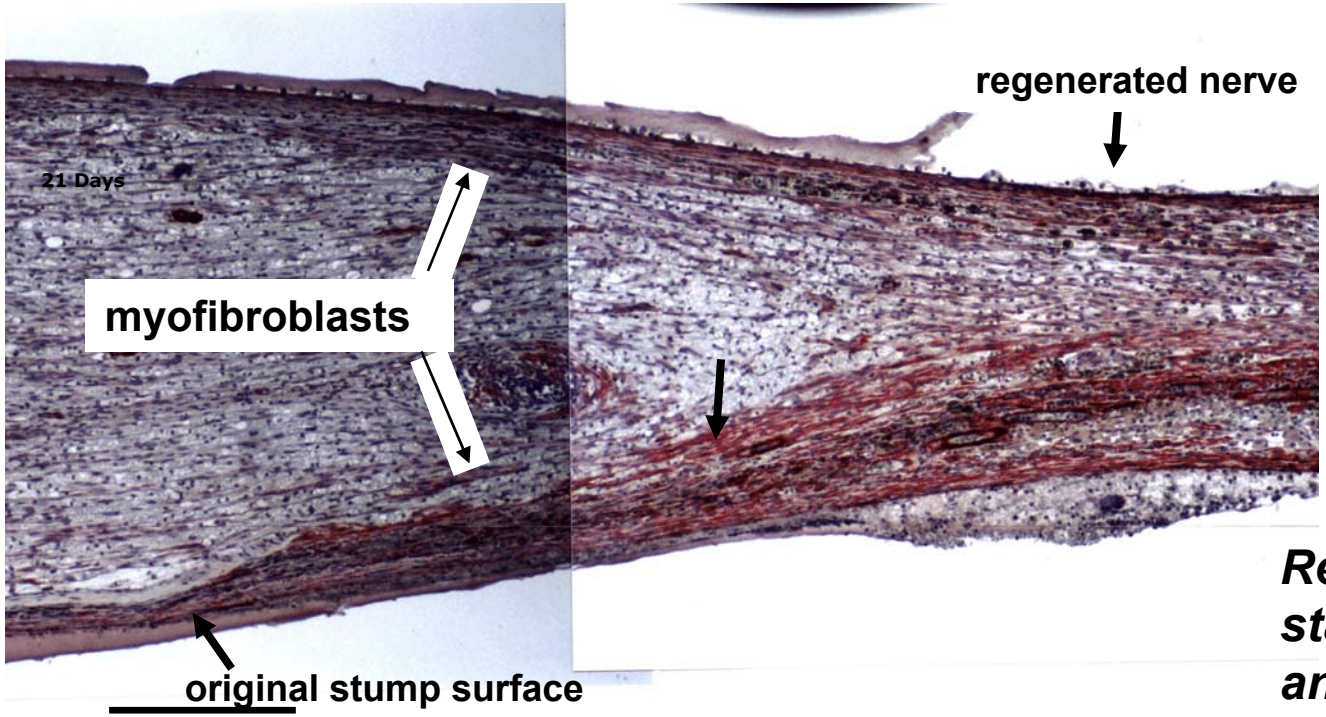
transected nerve stumps inside scaffold tube



The initial state of nerve wound healing studies

Inactive tube.
Thin regen-
erated nerve.

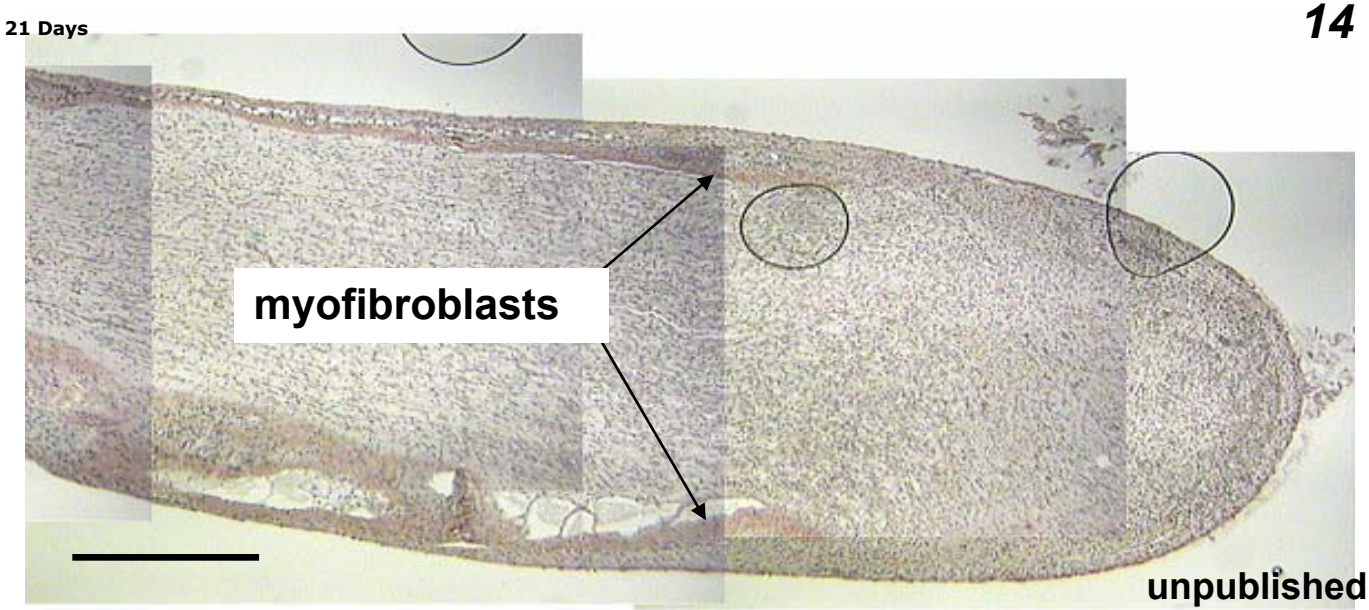
→



Red-brown: stained with antibody to α-SM actin. 14 d

No tube.
Neuroma.

→



Inactive scaffold tube

60 weeks

Photo removed for copyright reasons.

15-20 myofibroblast layers

Poor quality of nerve regeneration.

Nerve regeneration template (NRT) tube

60 weeks

Photo removed for copyright reasons.

1 myofibroblast layer

Superior nerve quality.

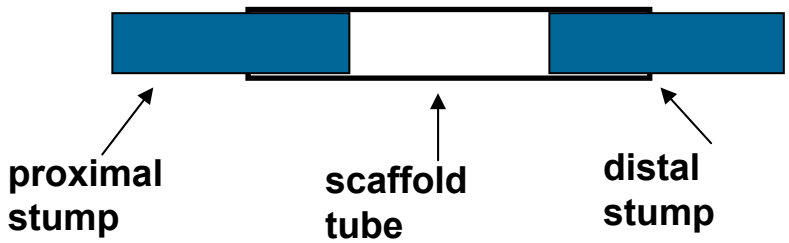
Red-brown: stained with antibody to α-SM actin. 60 weeks

Chamberlain et al., 2000

Well-regenerated nerve

Photo removed for copyright reasons.

NRT tube



Poorly regenerated nerve

inactive scaffold tube

Photo removed for copyright reasons.

Rat sciatic nerve transected. Stumps inserted in NRT tube or inactive tube.

Harley et al., 2003

Fact IV. 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 Facts I-IV.

I. During amphibian larval (tadpole) development;
also, during the fetal-to-adult transition in mammals:
C↑ R↓

II. As the severity of injury in an organ is increased from epithelia alone to epithelia with stroma:
C↑ R↓

III. 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

IV. Impaired healing blocks contraction but does not induce regeneration.
C = 0 R = 0

Contraction blockade theory explains the facts

symbols refer to [C, S, R]

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

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

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

Mechanism of contraction inhibition by DRT scaffold in skin wound

1. Fact: Reduction in number of myofibroblasts. Scaffold does not aggregate platelets. Collagen banding, but not triple helix, deliberately abolished in scaffold. Hypothesis: Early release of TGF-β is downregulated.
2. Fact: Disruption of myofibroblast organization. Myofibroblasts bind on scaffold extensively, chiefly via the $\alpha 1 \beta 1$ integrin. Hypothesis: myofibroblast contractile axes disoriented, cells separated; cell population loses its cohesion and force loses its uniaxial character.

Critical structural features of biologically ECM analogs used as scaffolds

1. chemical composition (ligand identity)

2. pore structure (ligand density)

4. macromolecular structure (scaffold duration)

Diagrams removed for copyright reasons.

3. orientation of pore channels (ligand spatial coordinates)

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

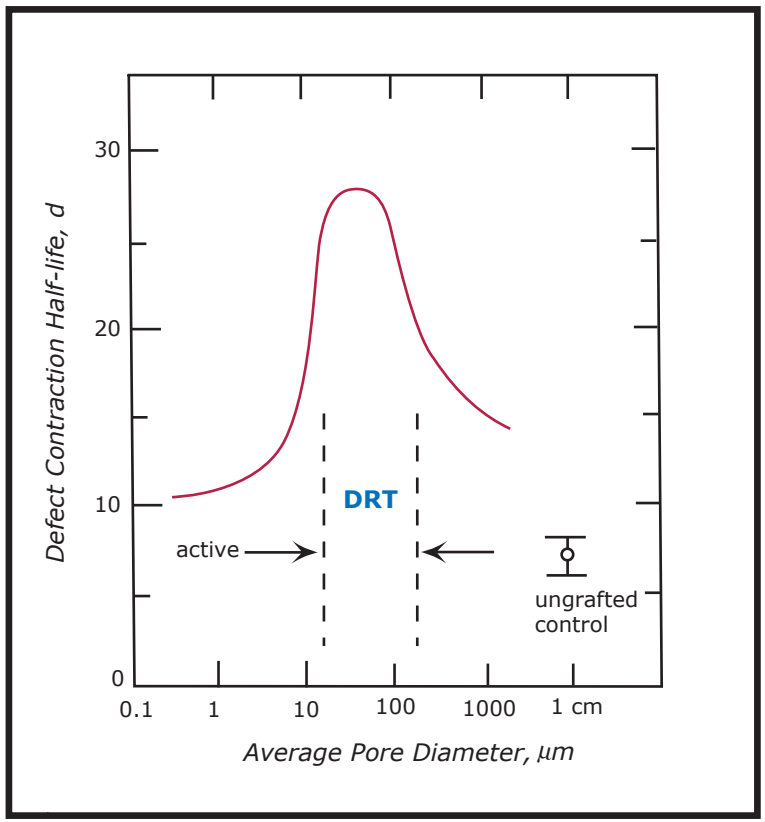


Figure by MIT OCW. After Yannas, 1989.

Yannas et al., 1989

Pore size vs bound cell density

- **The volume density ρ_c of cells is equal to the product of the specific surface of template, σ, and the surface density of bound cells, Φc:**
- $$\rho_c = \sigma \Phi_c$$
- **We will compare ρ_c for two types of scaffold, differing only in average pore size.**
- **Observations of myofibroblast density inside a template with average pore diameter of about 10 μm have yielded typical values of the volume density, ρ_c , of order 10^7 myofibroblasts per cm^3 porous template. For this template the specific surface σ is calculated, using a standard model for a porous solid with pore volume fraction of about 0.95, to be approximately $8x10^4$ mm^2/cm^3. The cell surface density is accordingly $\Phi_c = \rho_c/\sigma = 10^7/8x10^4 = 125$ cells/mm^2.**

- **With a template of identical composition but average pore diameter as large as 300 μm, Φ_c is the same as above since the two solids have identical types of ligands and ligand density; however, the specific surface is now calculated to be about 3×10^3 mm^2/cm^3 template. In this case, the volume density of cells is only $\rho_c = \Phi_c \sigma = 125 \times 3 \times 10^3 = 3.75 \times 10^5$ per cm^3 porous template. We conclude that the template with an average pore diameter of 10 μm binds a volume density of myofibroblasts which is about 27 times higher than does the template with a pore diameter of 300 μm. The density of ligands clearly drops as the pore diameter increases; eventually, the ligand density becomes insufficient to bind all or most of the myofibroblasts and contraction resumes, as observed.**
- **These considerations suggest a maximum pore diameter requirement for the template, simply to ensure a specific surface which is large enough to bind a number of myofibroblasts that is appropriately large to block contraction across the entire scale of the defect.**

Microscopic to macroscopic contraction force

- **Exerting each an individual contractile force vector, f_i , a number N of in-plane myofibroblasts scale up the force to the level of the macroscopic force vector, F_c, where wound contraction occurs:**

$$F_c = Nf_i$$

- **Measurement of the macroscopic force to contract a skin wound (in the absence of a template) yielded a force of order 0.1 N. Calculation of f_i with dermal fibroblasts bound *in vitro* on the DRT surface led to a value of order 1 nN per cell. Use of Eq. together with these measured values of the force per cell and the macroscopic force leads to an estimated**

  $N = 10^{-1}\text{N}/1\text{nN} = 10^8$ cells participating in contraction of the template-free injured site. This calculation suggests, therefore, an apparent force scale-up factor of 10^8 (cell$\rightarrow$wounded organ).

Reduction of contraction force by template

- **The overall effect of the template inside the wound is, therefore, to reduce the factor *N* and, separately, the factor f_i, in Eq. and thereby lead to a very significant drop in the macroscopic contraction force F_c to near zero.**

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. Synchronous and isomorphous replacement.

Synthesis of stroma

Isomorphous replacement

Replacement of template topography by a similar one.

Synchronous tissue synthesis

Synthesis of dermis (regeneration) was therefore observed when the time constants for template degradation and new tissue synthesis were approximately equal

$$t_d / t_h \cong 1$$

Summary of stroma synthesis. Synthesis occurs at a rate that matches the rate of template degradation and in a spatial configuration that replicates the departing template.

Dermis regeneration template (DRT)

Photo removed for copyright reasons.

100 μm

Yannas, 2004

Nerve regeneration template (NRT)

Photo removed for copyright reasons.

100 μm

Yannas, 2004

Similarity of configuration + synchronization of template degradation rate.

Fact #1: A scaffold with regenerative activity (templates) in skin, conjunctiva and peripheral nerves has a three-dimensional configuration (topology) that is very similar to that of the stroma in the regenerating organs (Dagalakis et al., 1980; Chang et al., 1990; Hsu et al., 2000).

Hypothesis: Organ regeneration proceeds on the surface of a matrix that is a replica of the native stroma of the organ.

Fact #2: Regeneration templates lose their activity unless their degradation rate is roughly equal to the timescale for synthesis of tissue during healing (Yannas et al., 1979: Yannas and Burke, 1980).

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

Isomorphous tissue replacement rule

Similarity of configuration + synchronization of degradation rate of template → Rule of isomorphous tissue replacement (Yannas, 1997):

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.

Explain facts of regeneration using unified theory:

Contraction blockade +

+ Synchronized and Isomorphous replacement →

→ Regeneration

E. Two theories of regeneration:

1. Immunocompetence theory.

2. Contraction blocking and synchronized isomorphous replacement.

Immunocompetence theory: Loss of regenerative potential with development of immune system (amphibian data).

Diagram removed for copyright reasons.

Harty et al., 2003

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