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A theory of induced regeneration in adults.

Note: [C, S, R] data only cited; no kinetics

Outline

1. Irreversible injury
2. Regenerative and nonregenerative tissues
3. Antagonistic relation between contraction and regeneration
4. Present theory: Selective inhibition of contraction necessary but not sufficient for regeneration
5. Mechanism

1. Irreversible injury

Reversible injury

Image removed due to copyright considerations.
See Figure 1.1 in Yannas, I. V. *Tissue and Organ Regeneration in Adults*. New York: Springer-Verlag, 2001.

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

Goss, 1992

Irreversible injury

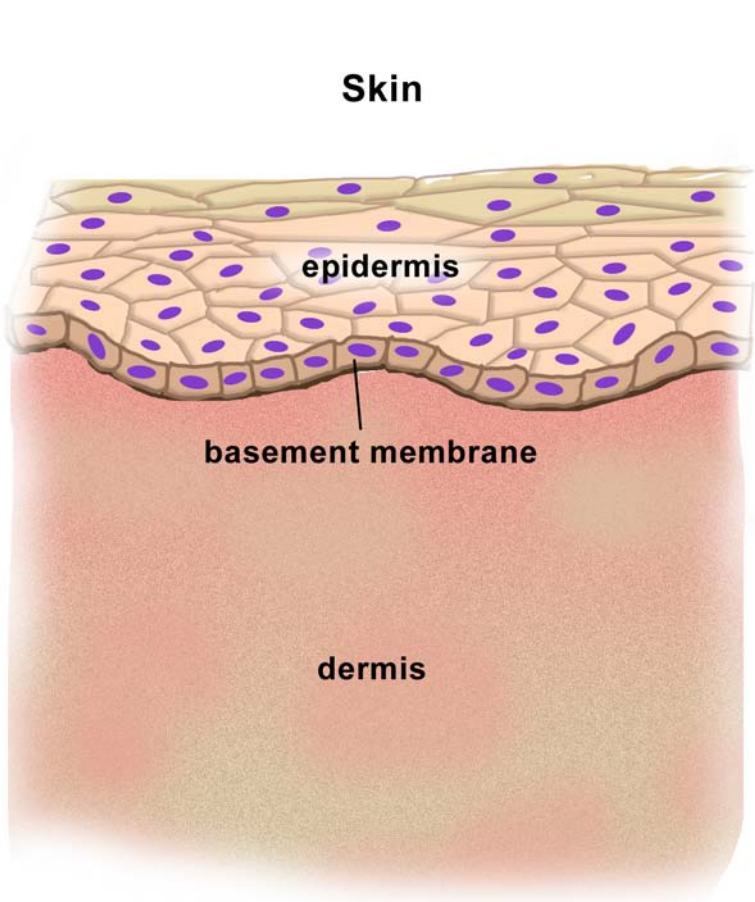
Image removed due to copyright considerations.

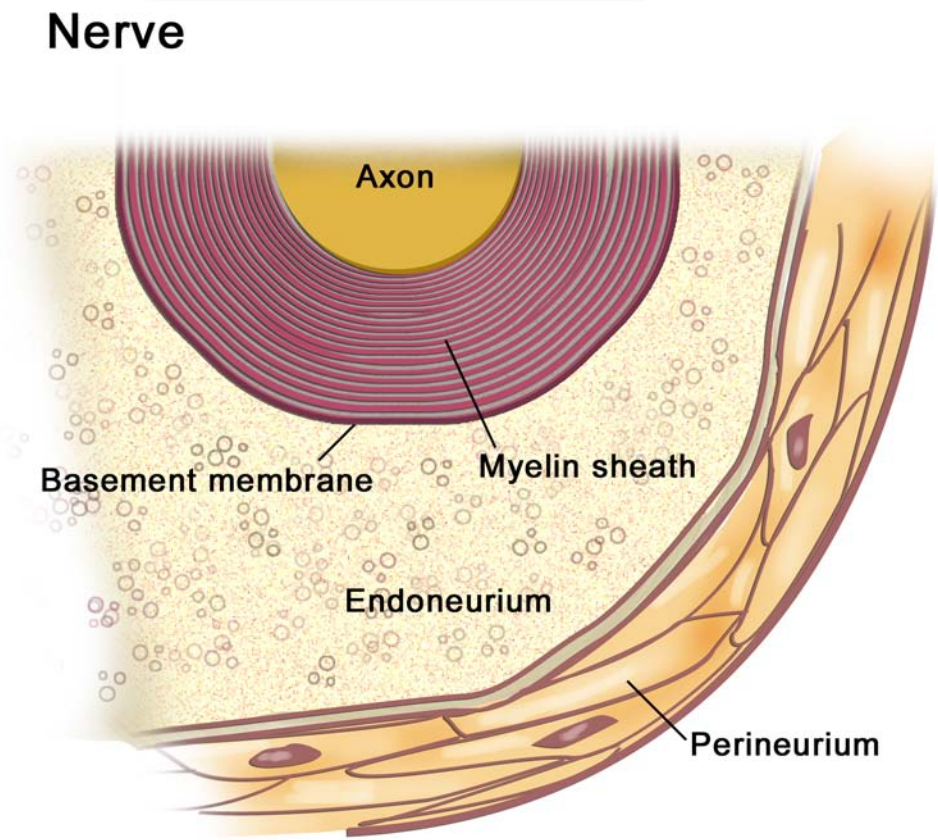
Burn victim suffering from severe contraction and scar formation

Tomasek et al., 2000

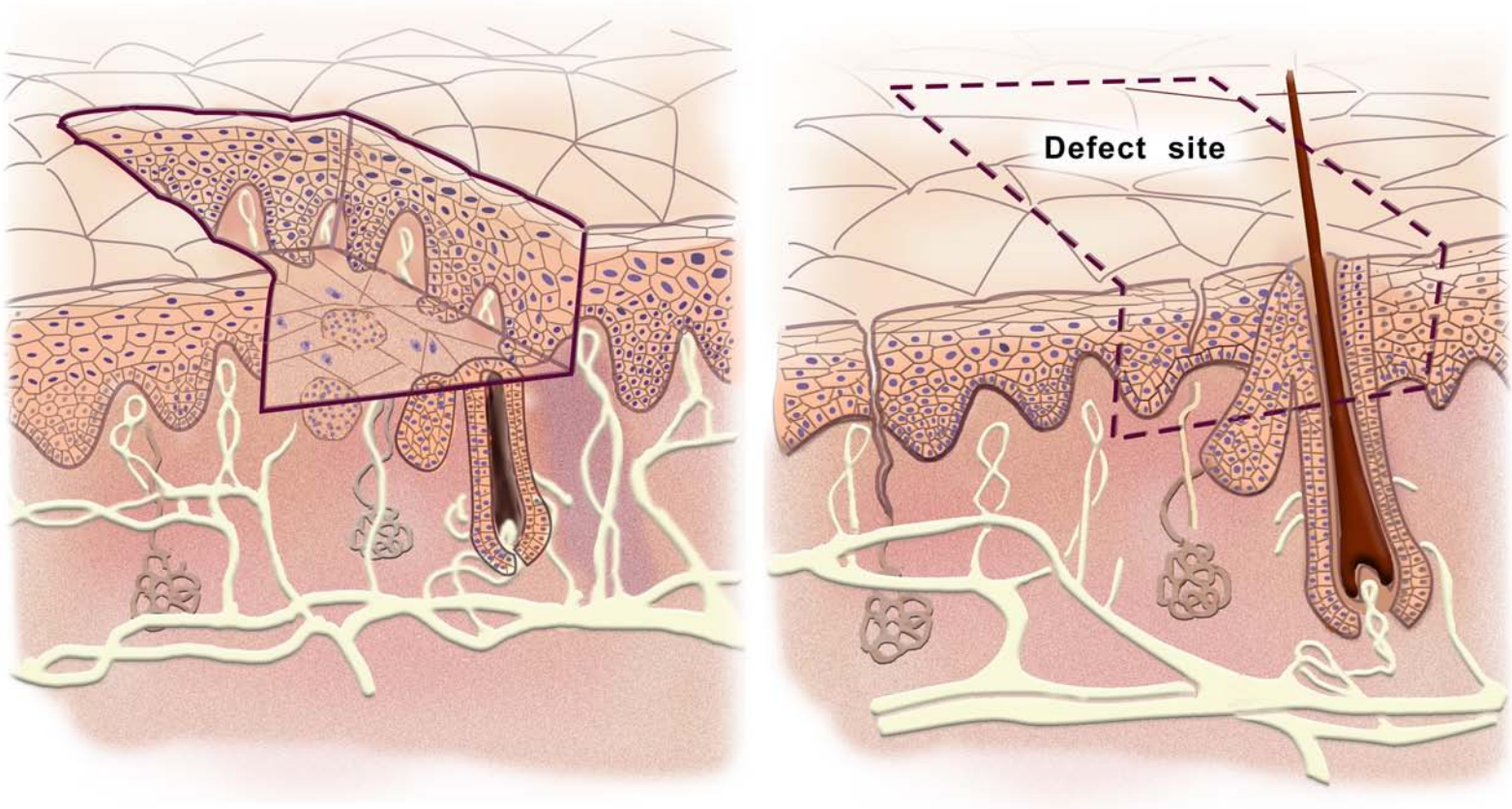
2. Regenerative and nonregenerative tissues in adult mammals

The tissue triad in skin and nerves



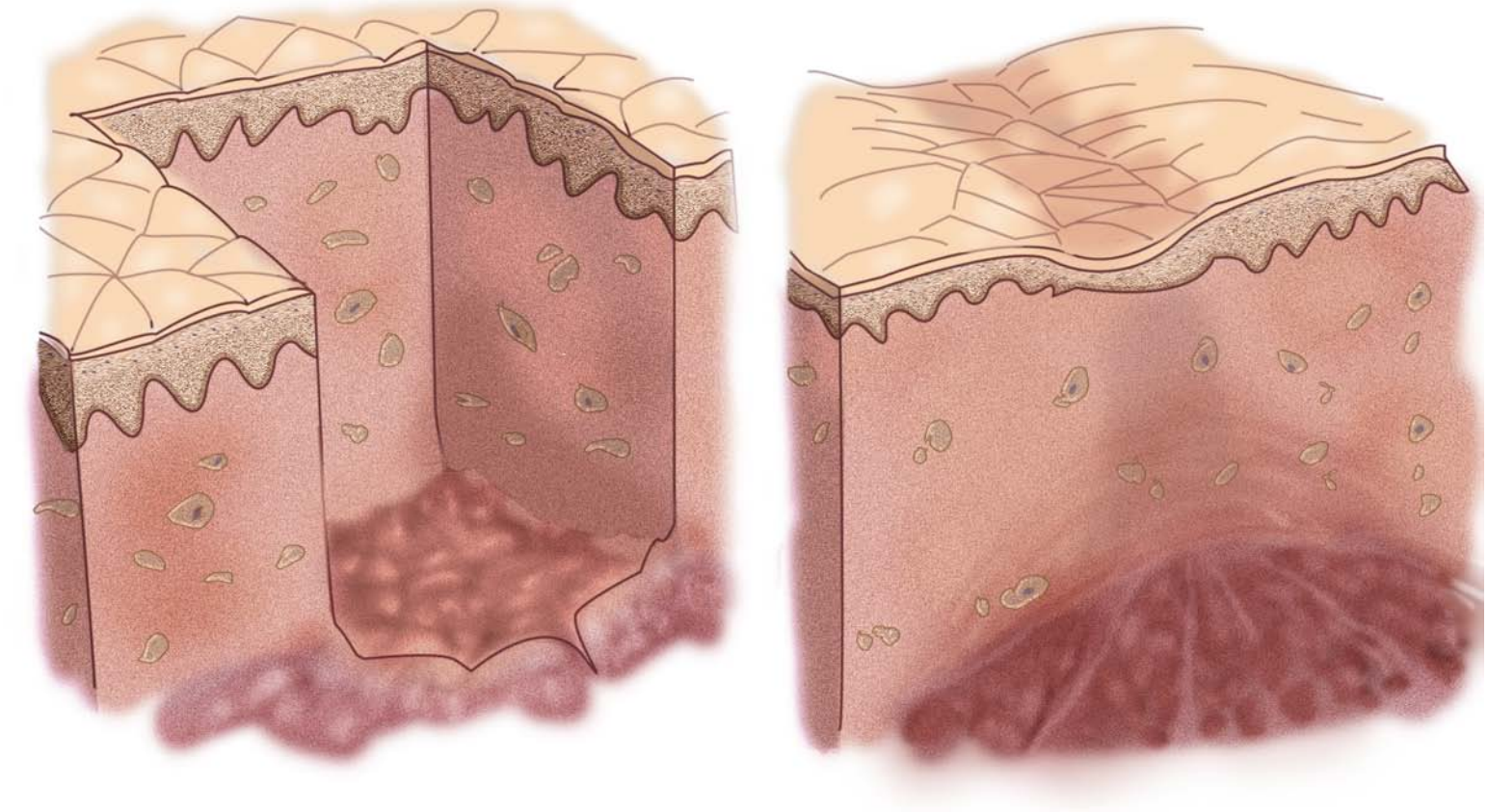


Skin



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

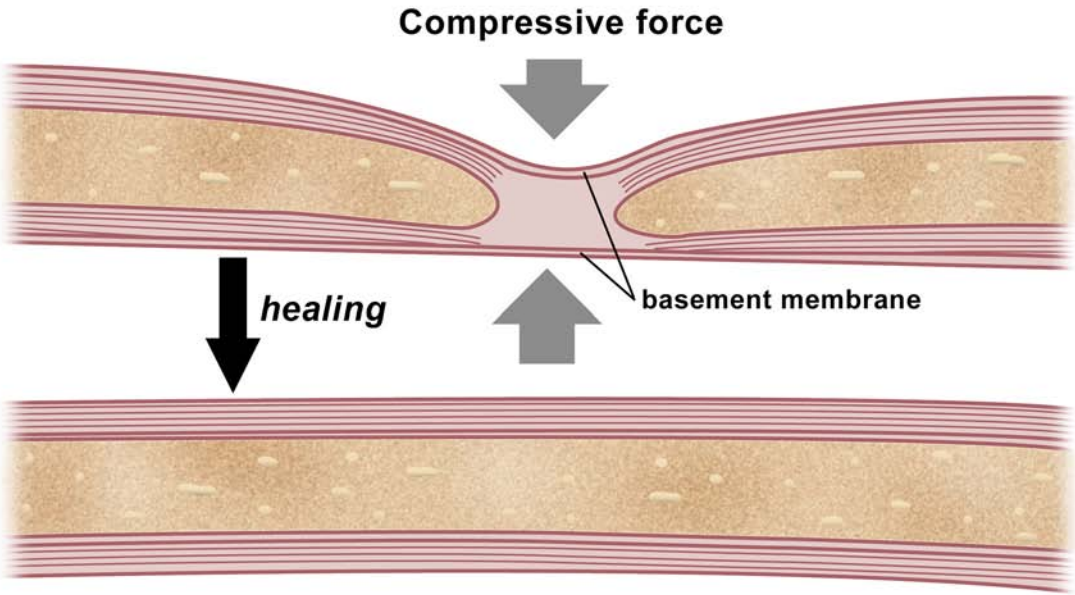
Skin



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

Peripheral nerve

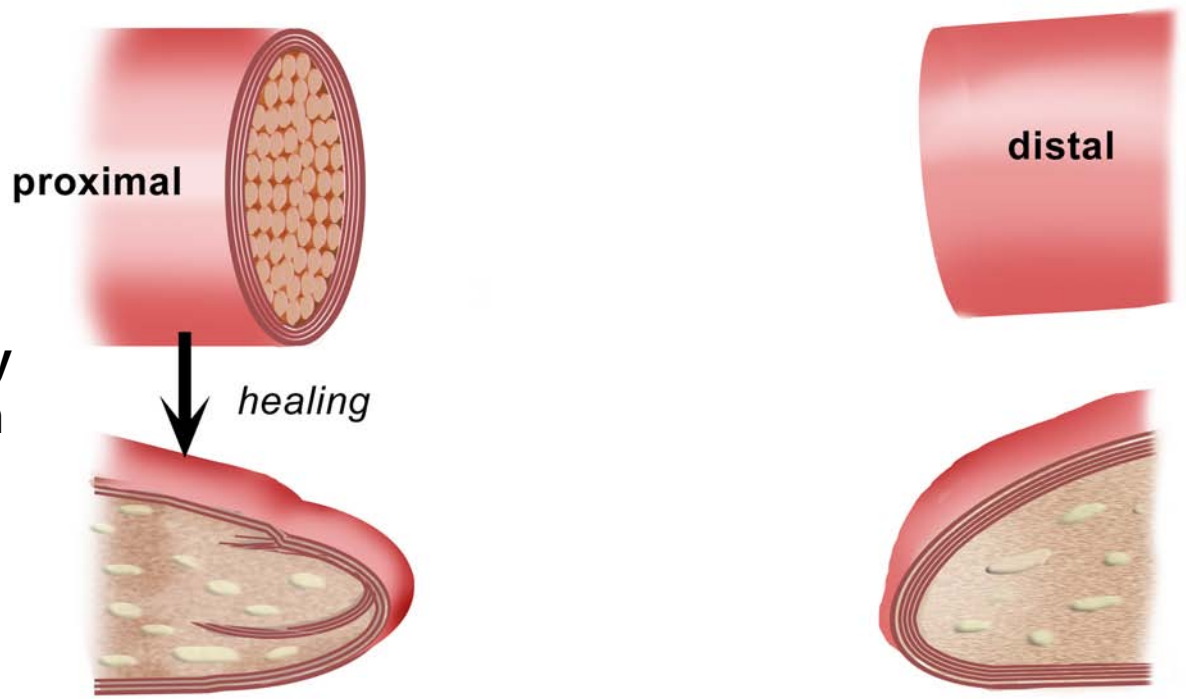
crushed nerve heals spontaneously by regeneration



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

Peripheral nerve

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. Contraction occurs.

SUMMARY SO FAR

	Regenerative tissues. Reversible injury. No contraction.	Nonregenerative tissues. Irrever-sible injury. Contraction +scar.
SKIN	epidermis	dermis
	BM	
NERVE	myelin	endoneurial stroma
	BM	

Conclusion: Inverse relation between contraction and regeneration

- During adult healing, no contraction is observed following injury to epithelia or basement membrane.
- Contraction is only observed following injury to stroma.
- Contraction only observed following irreversible injury.

3. Antagonistic relation between contraction and regeneration

a. Data from spontaneously healing wounds
b. Blocking of contraction using scaffolds
c. Isolation of contraction during “island” grafting
d. Scar formation vs inhibition of contraction
e. Contraction during impaired healing

Quantitative description of healing processes

- **Initial wound area is A_o**
- **Wound eventually closes up spontaneously. Final area is A_f.**
- **Final wound area is distributed among fractions that closed by contraction (%C), scar formation (%S) or regeneration (%R).**
- **This is the configuration of the final state.**
- **Wound closure rule:**

$$C + S + R = 100$$

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]**

a. Data from spontaneously healing wounds

Tadpole → Frog

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

(early stages) → (late stages)

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

tadpole → frog

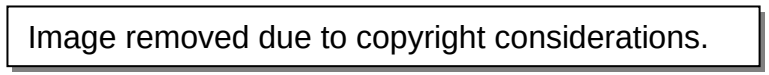
b. Blocking of contraction using scaffolds

Organ/ species	Treatment used	Spontan-eous	Treated
skin-guinea pig	scaffold A	[91, 9, 0]	[89, 0, 11]
skin-guinea pig	scaffold DRT+ KC	[92, 8, 0]	[28, 0, 72]
nerve-rat	silicone tube+scaf-fold DRT	[95, 5, 0]	[53, 0, 47]
nerve-rat	collagen tube+scaf-fold NRT	[95, 5, 0]	[0, 0, 100]
conjunctiva -rabbit	scaffold DRT	[45, 55, 0]	[13, 0, 87]

Skin

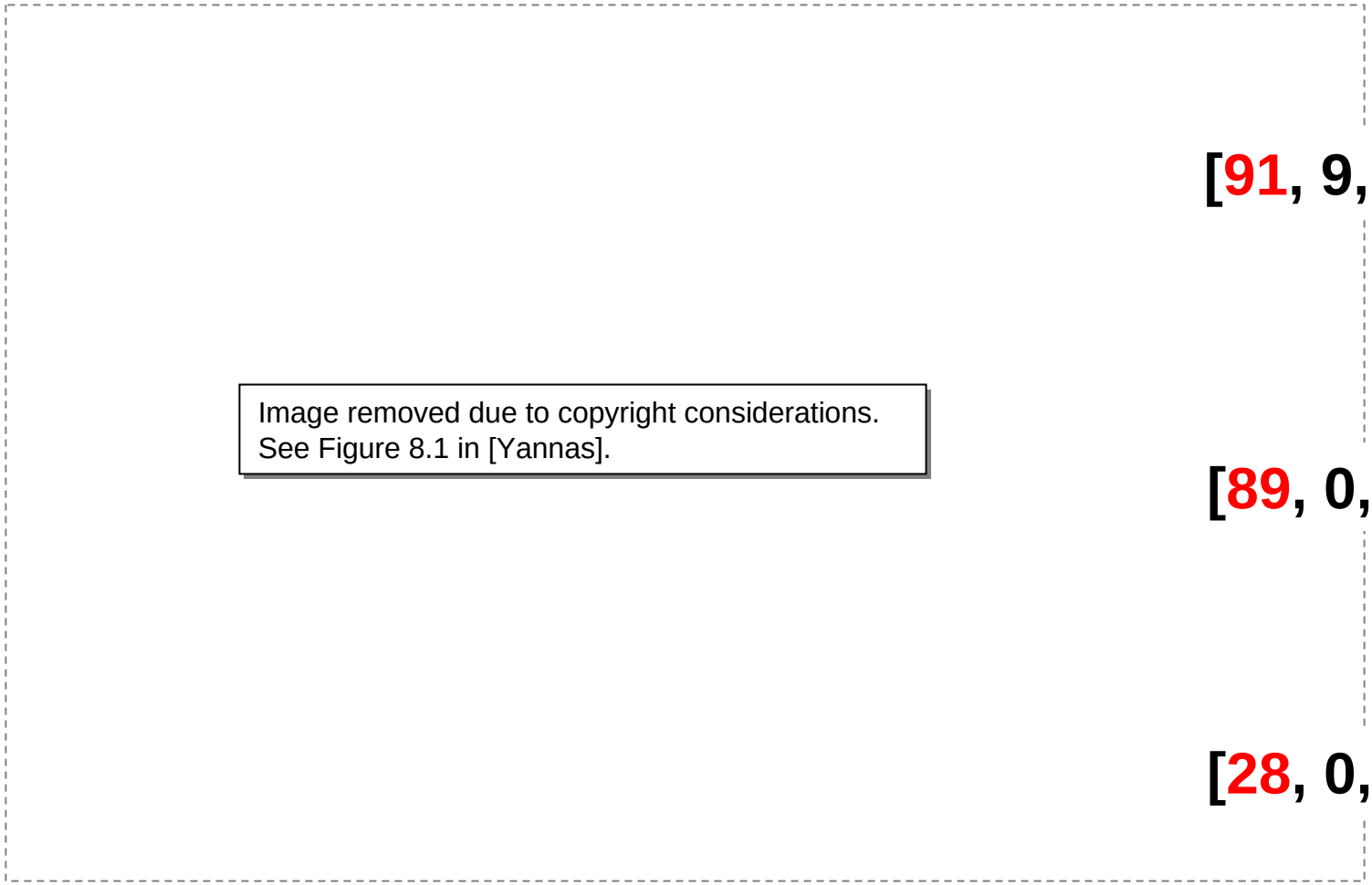
Skin

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



Skin

Wound area closure using three protocols



Dermis regeneration template (DRT)

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Structural features of biologically ECM analogs

1. chemical composition (ligand identity)

2. macromolecular structure (ligand duration)

3. pore structure (ligand density)

Diagrams removed due to copyright considerations.

4. orientation of pore channels (ligand orientation)

Contraction inhibited maximally in pore diameter range 20 μm — 120 μm. Scaffolds with pores in that range induced dermis regeneration. Scar formed outside that range.

Image removed due to copyright considerations.
See Figure 8.5 - top in [Yannas].

DRT

Normal skin

rete ridges with capillary loops and vascular plexus underneath

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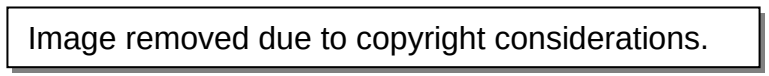
See Figure 5.2 (top left) in [Yannas].

Burkitt et al., 1992

Verify induced regeneration of skin basement membrane.
I: Immunostaining: Factor VIII for capillary loops



75 μm

Compton et al., 2000

Verify induced regeneration of skin basement membrane.
II. Immunostaining: $\alpha_6\beta_4$ Integrin for hemidesmosomes

Image removed due to copyright considerations.

100 μm

Compton et al., 2000

Verify induced regeneration of skin basement membrane.
III. Immunostaining: Collagen VII for anchoring fibrils

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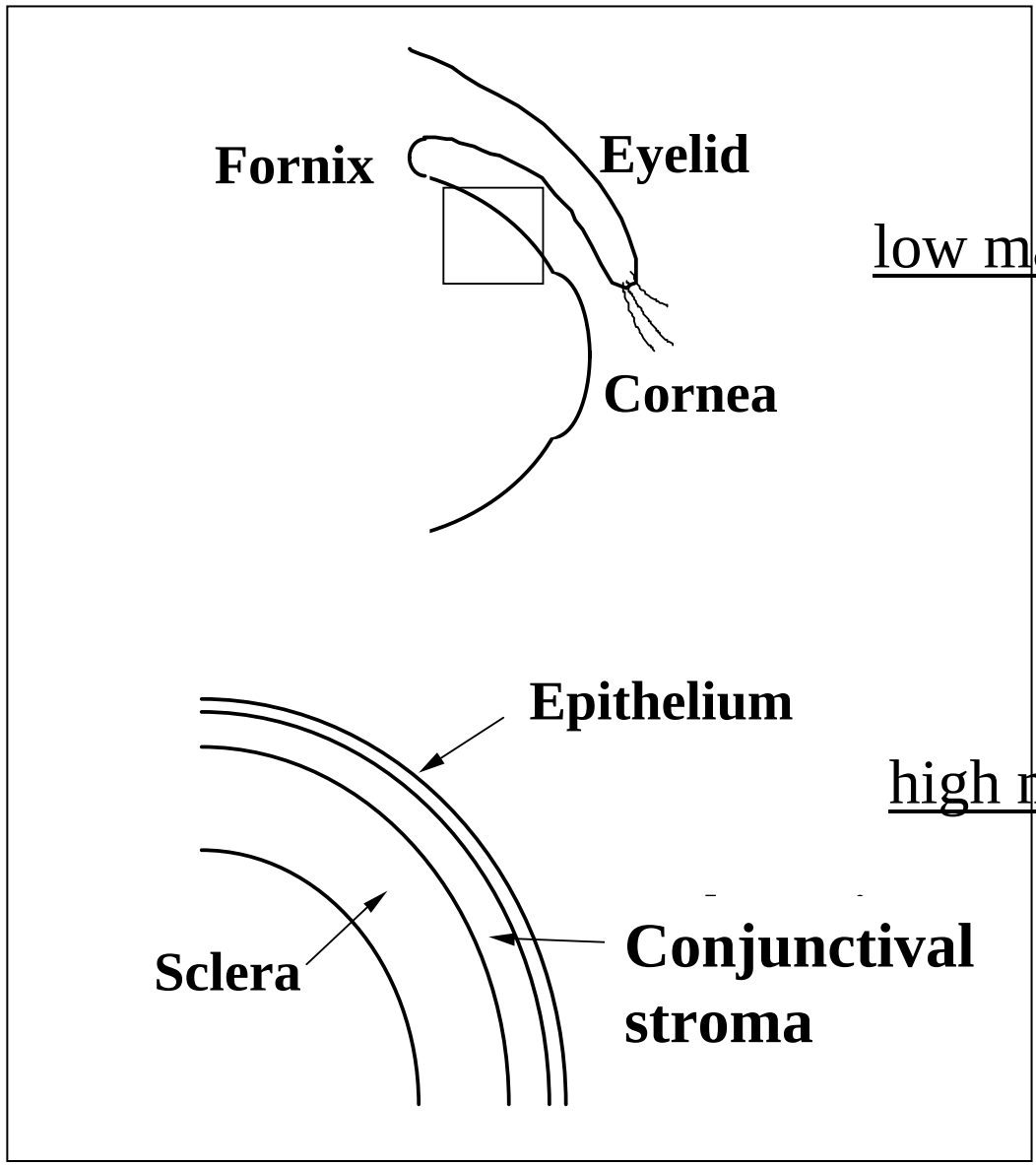
150 μm

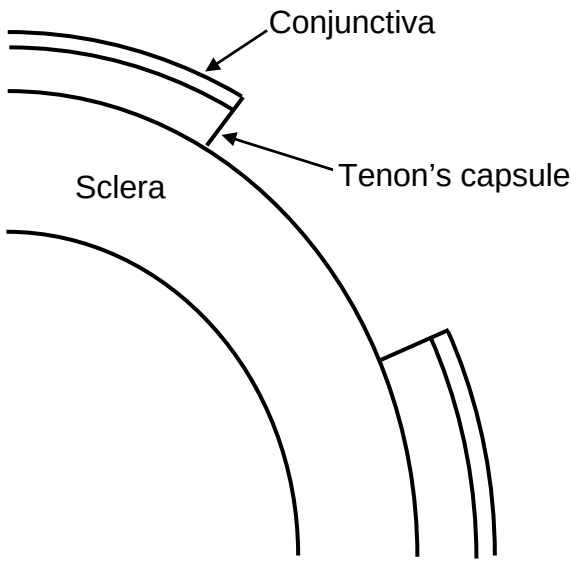
Compton et al.,2000

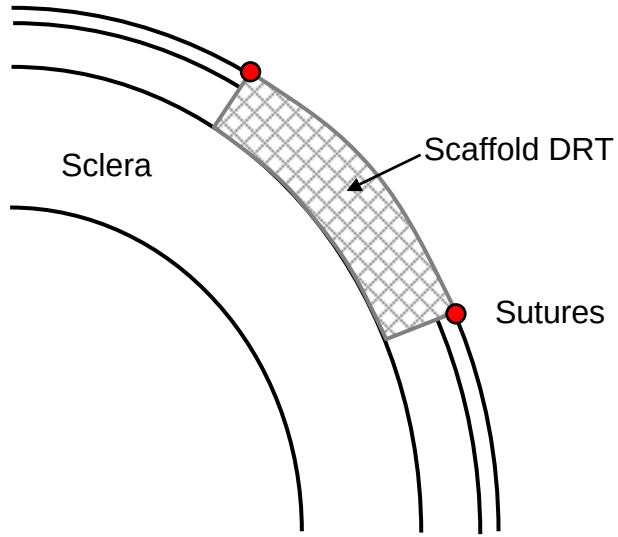
Conjunctiva

Anatomy of the conjunctiva

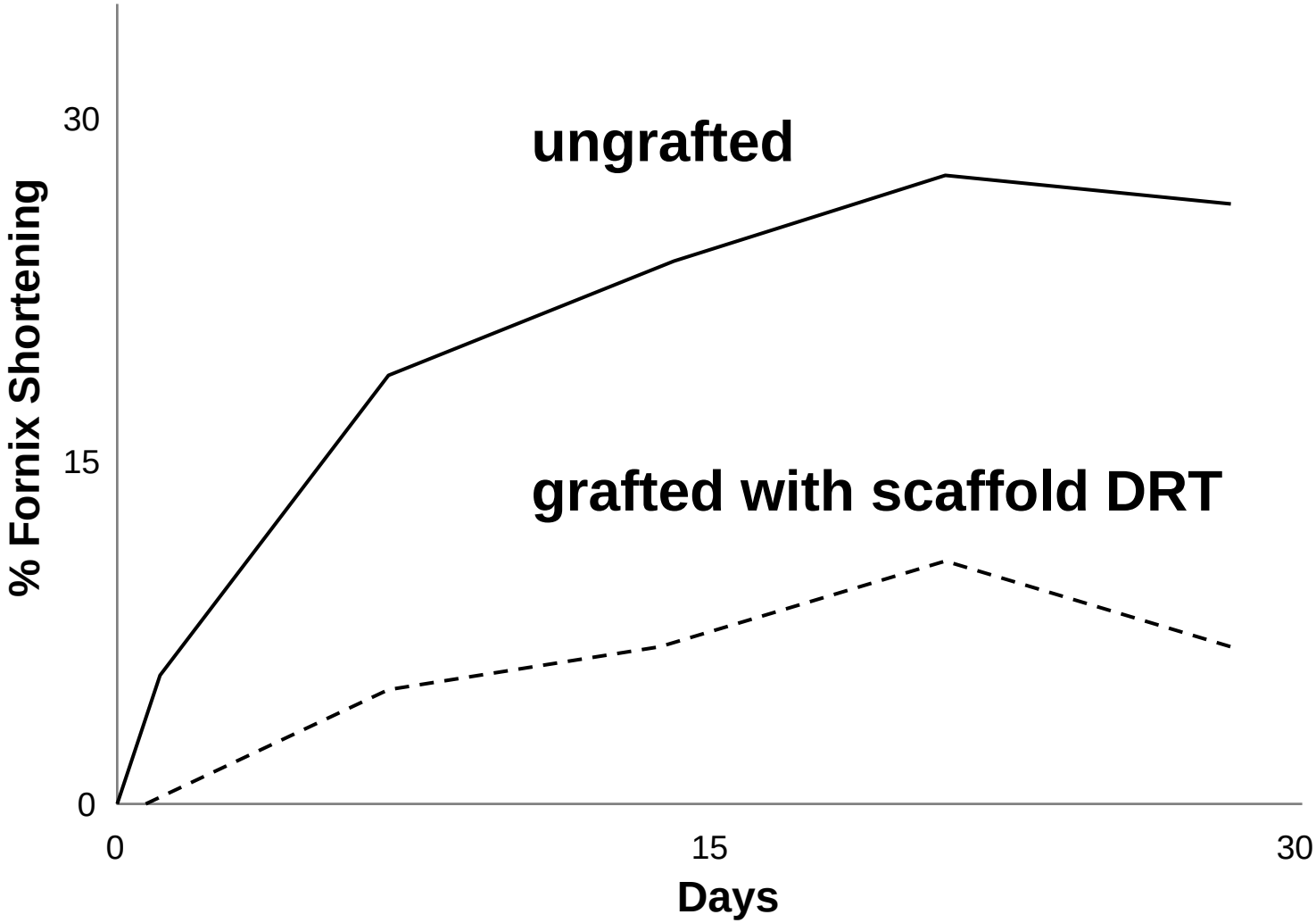


Conjunctiva wound model





Effect of DRT on contraction kinetics of conjunctival defect. It is experimentally convenient to study contraction of the fornix, a tissue attached to the conjunctiva.



Hsu et al., 2000

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

ungrafted conjunctival scar

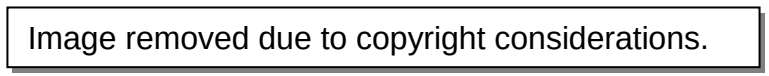
grafted with scaffold DRT

normal conjunctiva

Hsu et al., 2000

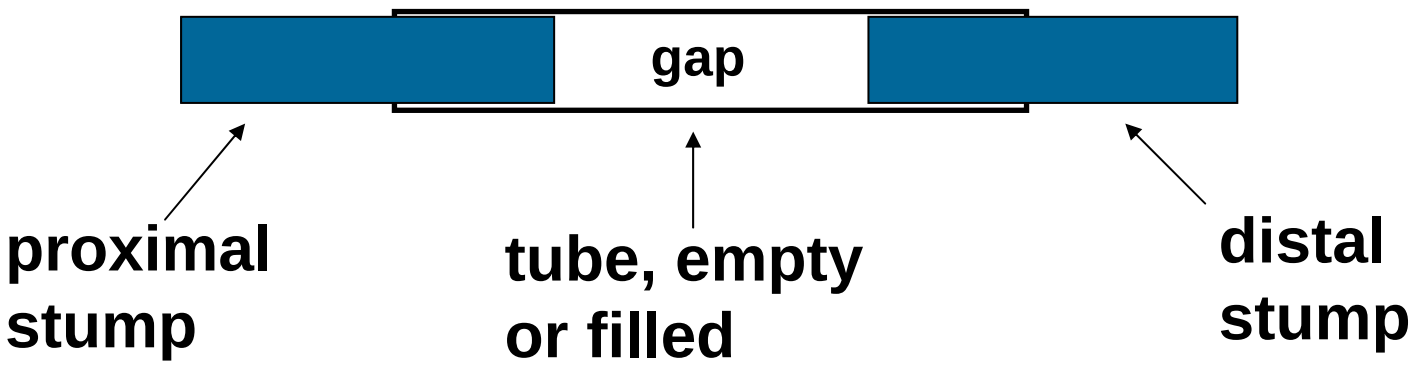
Peripheral nerve

Rat sciatic nerve model



Experimental model used to study PNS regeneration

- Transect rat sciatic nerve
- Insert nerve stumps into tube
- “Nerve chamber” model is standard



Nerve regenera-ted across 8-mm gap

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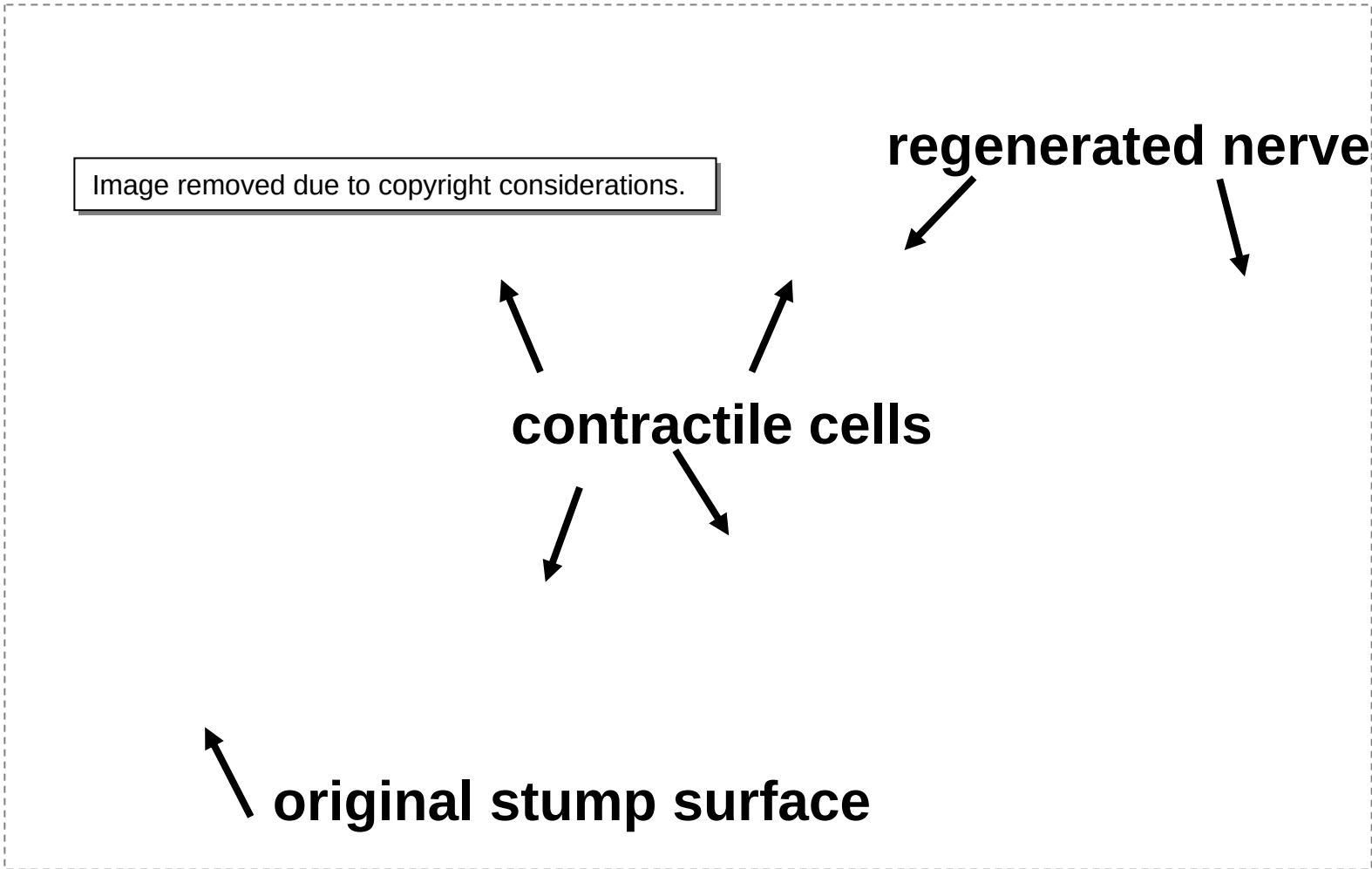
See Figure 10.7 (lower right) in [Yannas].

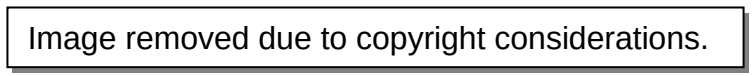
Jenq and Coggeshall, 1985

Contractile cell zone surrounds regenerating nerve



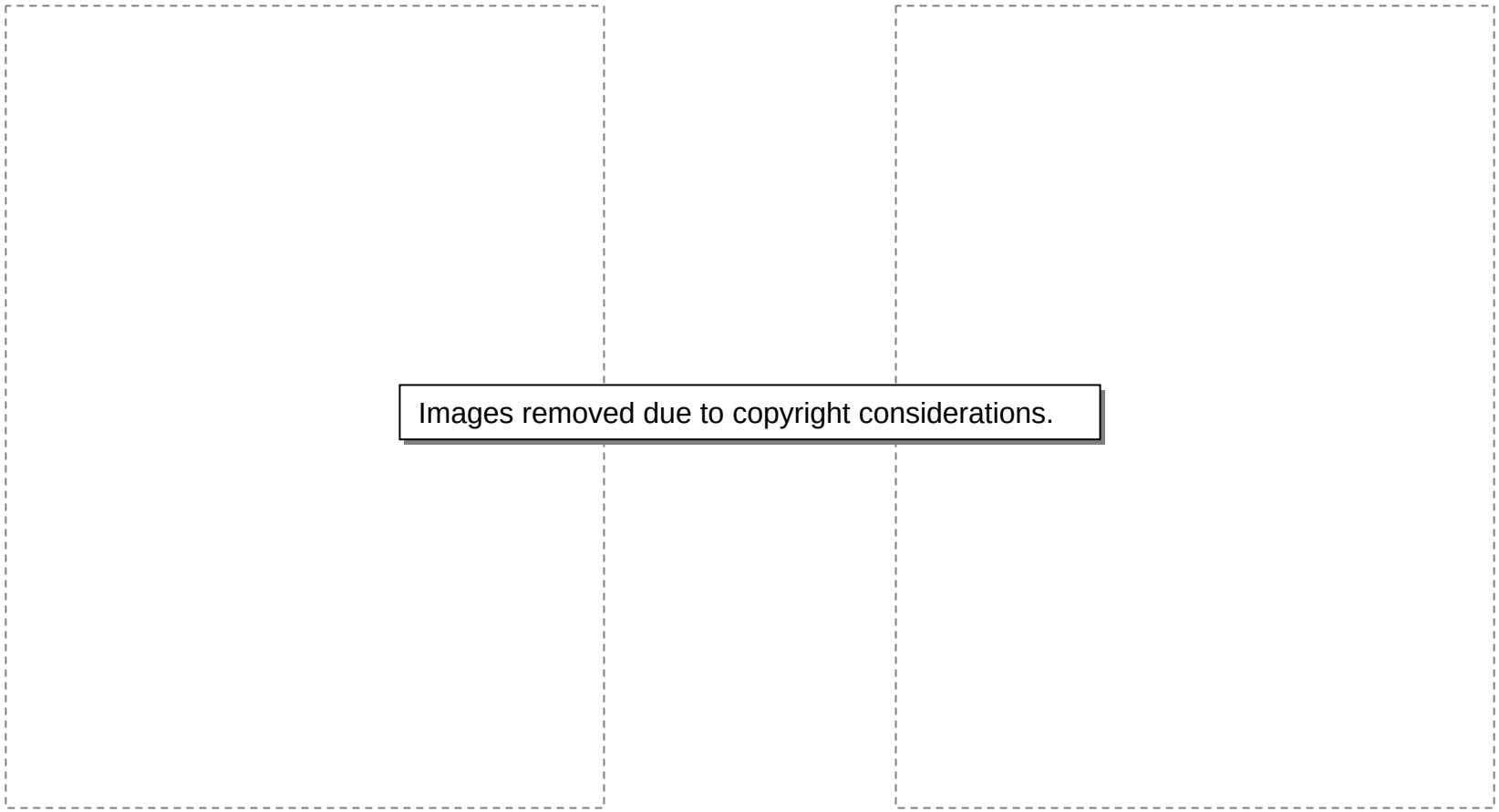
Spilker and Seog, 2000

Nerve regeneration template (NRT)



silicone tube

scaffold tube



15-20 contractile cell layers
poor quality of regeneration

0-1 contractile cell layer
superior quality

Chamberlain et al., 2000

c. Scar formation vs inhibition of contraction

Data in table will show: Inhibition of contraction, even modest, leads to virtual abolition of scar

Scar appears to be a by-product of contraction

Organ/ species	Treatment used	Spontan-eous	Treated
skin-guinea pig	scaffold A	[91, 9, 0]	[89, 0, 11]
skin-guinea pig	scaffold DRT+ KC	[92, 8, 0]	[28, 0, 72]
nerve-rat	silicone tube+scaf-fold DRT	[95, 5, 0]	[53, 0, 47]
nerve-rat	collagen tube+scaf-fold NRT	[95, 5, 0]	[0, 0, 100]
conjunctiva -rabbit	scaffold DRT	[45, 55, 0]	[13, 0, 87]

d. 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 not regeneration

4. Theory: Selective inhibition of contraction

Conclusions

1. During development, contraction increases in importance, while regeneration corespondingly decreases (*rana catesbeiana*).
2. Certain scaffolds block contraction "selectively" and induce partial regeneration in adult mammals (rodents, swine, human).
3. Scar is abolished when contraction is blocked.
4. Impaired healing blocks contraction but does not induce regeneration.

Theory

refers to [C, S, R]

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

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

Mechanism of contraction inhibition by DRT scaffold in skin wound

- **Scaffold does not aggregate platelets (during its preparation, abolish collagen banding but not triple helix). Hypothesis: Downregulate release of TGF-β.**
- **Scaffold binds TGF- β1 avidly (but nonspecifically). Hypothesis: Downregulate soluble cytokine concentration.**
- **Scaffold binds myofibroblasts extensively. Hypothesis: myofibroblast contractile axes disoriented, lose vectorial character.**
- **Scaffold competitively inhibits natural ECM**

Myofibroblast

Image removed due to copyright considerations.

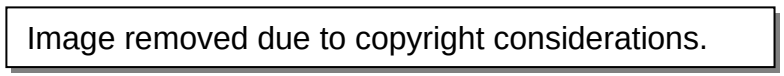
Tomasek et al., 2000

Myofibroblasts stain brown-red. Scaffold unstained.

surface of wound

A. No scaffold.
Contracting wound

surface of wound



B. Grafted with scaffold.
No contraction

100 μm

Kidney

Test applicability of theory to a new organ

Rat kidney

fibrotic tissue stains blue

Image removed due to copyright considerations.

untreated

scar formation and contraction of perimeter

Image removed due to copyright considerations.

treated with scaffold DRT

significantly smaller scar and less contraction of perimeter