

Skin synthesis in vivo (induced regeneration)*

- 1. Experimental parameters**
- 2. Synthesis of epidermis and BM**
- 3. Synthesis of dermis**
- 4. Partial synthesis of skin**
- 5. Comparative regenerative activity of reactants**
- 6. Overall mechanism of skin regeneration**

* *Tissue and Organ Regeneration in Adults*, Yannas, Springer, 2001, Ch. 5.

1. Experimental parameters (skin)

A. Anatomically well-defined defect

- Designate experimental volume**
- Delete nonregenerative tissue(s)**
- Anatomical bounds**
- Containment of exudate**

B. Timescale of observations

- Initial state: defect generated**
- Final state: defect closed**

Note: Remodeling continues after closure

Figure removed due to copyright restrictions.

See Figure 3.1 in [TORA].

Diagram with four types of anatomically well-defects for study of induced skin regeneration.

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

[Preview in [Google Books](#)]

1. Experimental parameters (skin) [Cont.]

C. Assays of final state (product of synthesis)

- Literature describes several assays, unrelated to nature of product synthesized (e.g., time of closure by epithelialization, % take of graft, ability to cross histocompatibility barriers).**
- Required assays are both qualitative (which tissue was synthesized?) and quantitative (How much?)**

2. Synthesis of an epidermis

Structure. Five cell layers (strata); 100 μm thick. Basal layer is closest to BM...stratum corneum is farthest out. Cell maturation gradient (increasing keratin content away from BM). Tissue turns over every 25-50 days.

Function. Protection against dehydration and microorganisms (primarily stratum corneum). Also protection against mechanical, thermal, chemical, UV insults.

Schematic view of epidermis

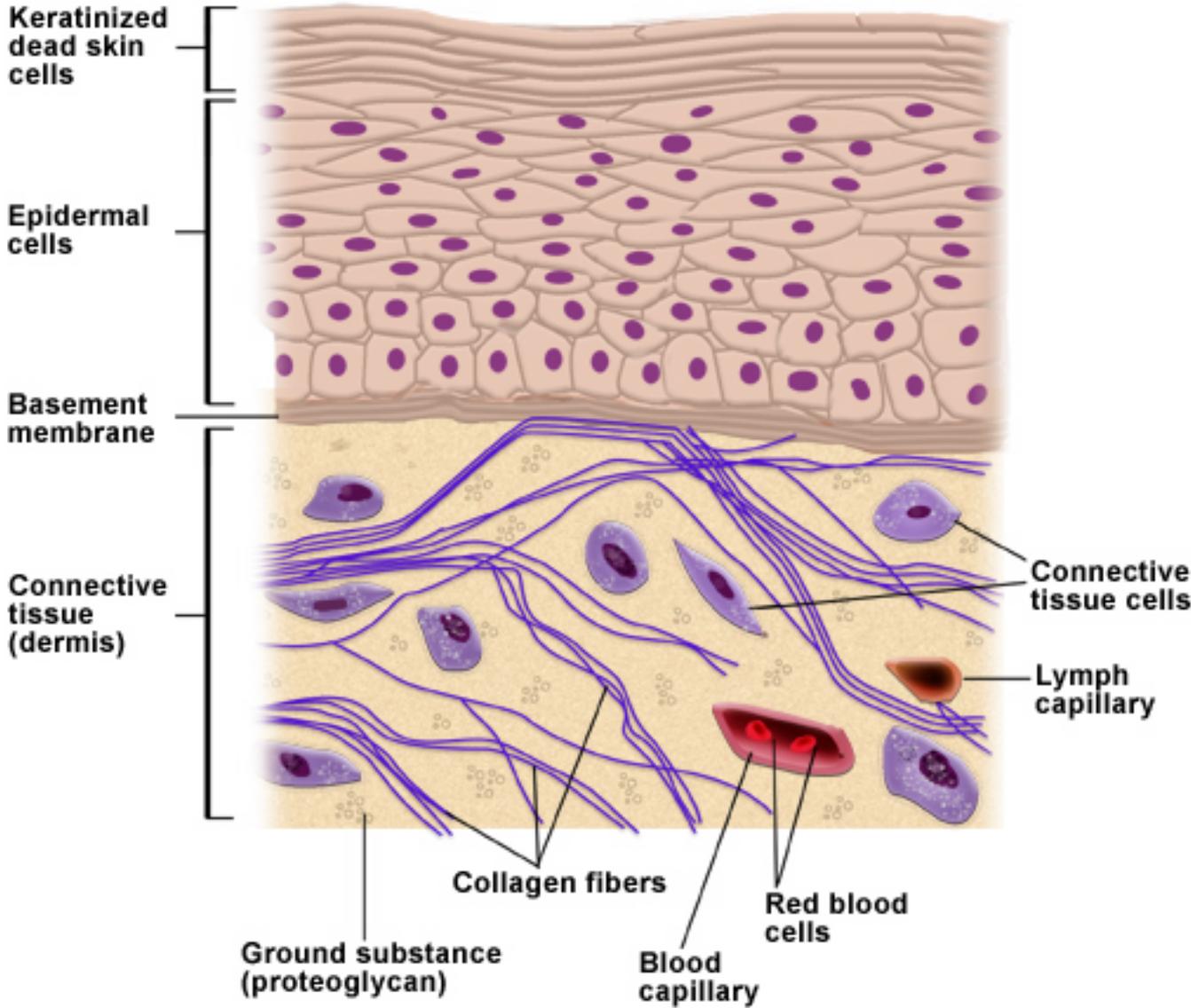


Figure by MIT OpenCourseWare.

2. Synthesis of an epidermis [Cont.]

Synthesis in vitro. Epidermis \Rightarrow trypsinization \Rightarrow dissociated keratinocytes (KC).

Condensation of KC to epidermis requires nondiffusible substrate (e.g., plastic surface) but not growth factors or dermal substrate.

In vivo. Epidermis synthesized spontaneously by KC, originally at the defect edge. KC dissociate spontaneously, migrate over residual dermis toward “center” of defect, synthesize BM and reform epidermis.

2B. Synthesis of BM

Structure. BM structure similar in all organs. 100 nm thick. Egg-carton topology in skin. Layer closest to epidermis is 20-40 nm thick (lamina lucida; mostly laminin). Intermediate layer 40-50 nm thick (lamina densa; type IV collagen). Next to stroma is fibroreticular layer (anchoring fibrils based on type VII collagen) that connects with type I collagen fibers in dermis via anchoring plaques. Hemidesmosomes connect basal cells to BM (tonofilaments).

**injury mode
(blister)**

**through epidermis:
reversible healing**

**between epidermis
and dermis:
reversible healing**

**through dermis:
irreversible healing**

Figure removed due to copyright restrictions.
See Figure 2.6 in [TORA].
Diagram showing regeneration of the basement
membrane after blistering.

Skin basement membrane

Figure removed due to copyright restrictions.
See Figure 5.1 (top) in [TORA].
Electron micrograph showing the two major
layers of the basement membrane.

**LL, lamina
lucida
LD, lamina
densa
d, dermis**

Skin BM

Figure removed due to copyright restrictions.
See Figure 5.1 (bottom) in [TORA].
Diagram showing the structure of the intact
basement membrane in skin.

2B. Synthesis of BM [Cont.]

Function. Boundary restricting transfer of cells and molecules; anchorage matrix for epithelial cells; mechanically competent “adhesive” layer binding epithelia to stroma; possibly “scaffold” facilitating repair after injury.

2B. Synthesis of BM [Cont.]

Synthesis in vitro. KC cultures in serum-free medium are transferred to solid surface. BM minus anchoring fibrils is synthesized.

In vivo. KC sheets are grafted on dermis-free defect; however, synthesized BM minus anchoring fibrils does not adhere to underlying tissue.

Complete BM formed when cultured KC sheets are grafted on dermis.

2B. Synthesis of BM [Cont.]

Mechanical failure of dermal-epidermal junction.

- 1952-56 Billingham et al. Epidermal sheets or KC suspensions grafted on dermis-free surface failed to adhere (“avulsion”).**
- 1977 Rheinwald and Green (RG) achieved KC culture expansion to KC sheets by 10,000X in 3 weeks.**
- 1980-95 Clinical studies of KC sheets prepared by RG method were terminated after completing 105 of them. Problem: avulsion of KC sheets from muscle substrate.**
- 1988-95 Woodley, Grinnell, Carver, Cooper et al. identified source of failure: lack of integration of BM to muscle substrate.**

3. Synthesis of dermis

Structure. Consists of two layers: Papillary dermis just below epidermis, comprising loosely packed, thin, type I collagen fibers, as well as dermal papillae with vascular loops and nerve endings. Reticular dermis comprises closely packed, thicker, type I collagen fibers; also elastin fibers.

Mechanically robust tissue comprises two interpenetrating networks of stiff, crystalline collagen fibers and extensible, amorphous, elastin fibers.

3. Synthesis of dermis (Cont.)

Function. Supports epidermis.

- tough base absorbs mechanical forces.**
- rich vascular network supports metabolically the avascular dermis.**
- thermoregulatory control for organism (sweat glands).**
- tactile, pain, hot/cold sensation, “love” nerve sensations.**

3. Synthesis of dermis (Cont.)

Synthesis in vitro. Not observed.

Synthesis in vivo via sequential synthesis of dermis and epidermis. Graft biologically active ECM analog (dermis regeneration template, DRT) on muscle substrate to synthesize dermis. Later, KC from defect margin migrate inside defect and synthesize BM and epidermis. (also via simultaneous synthesis---see below)

4. Partial synthesis of skin

Structure. Largest organ (about 18% body weight). Epidermis bonded to dermis with “rete ridges” (egg-carton topology). Elderly lack rete ridges; their skin peels off easier; mechanical and metabolic role of rete ridges; not present in scar).

Function. 1. Prevents dehydration and invasion of bacteria and viruses. 2. Largest sensory organ, contains receptors for touch, pressure, pain, temperature. 3. Helps thermoregulate body (controls heat transfer). 4. Major source of vitamin D supply.

4. Partial synthesis of skin [Cont.]

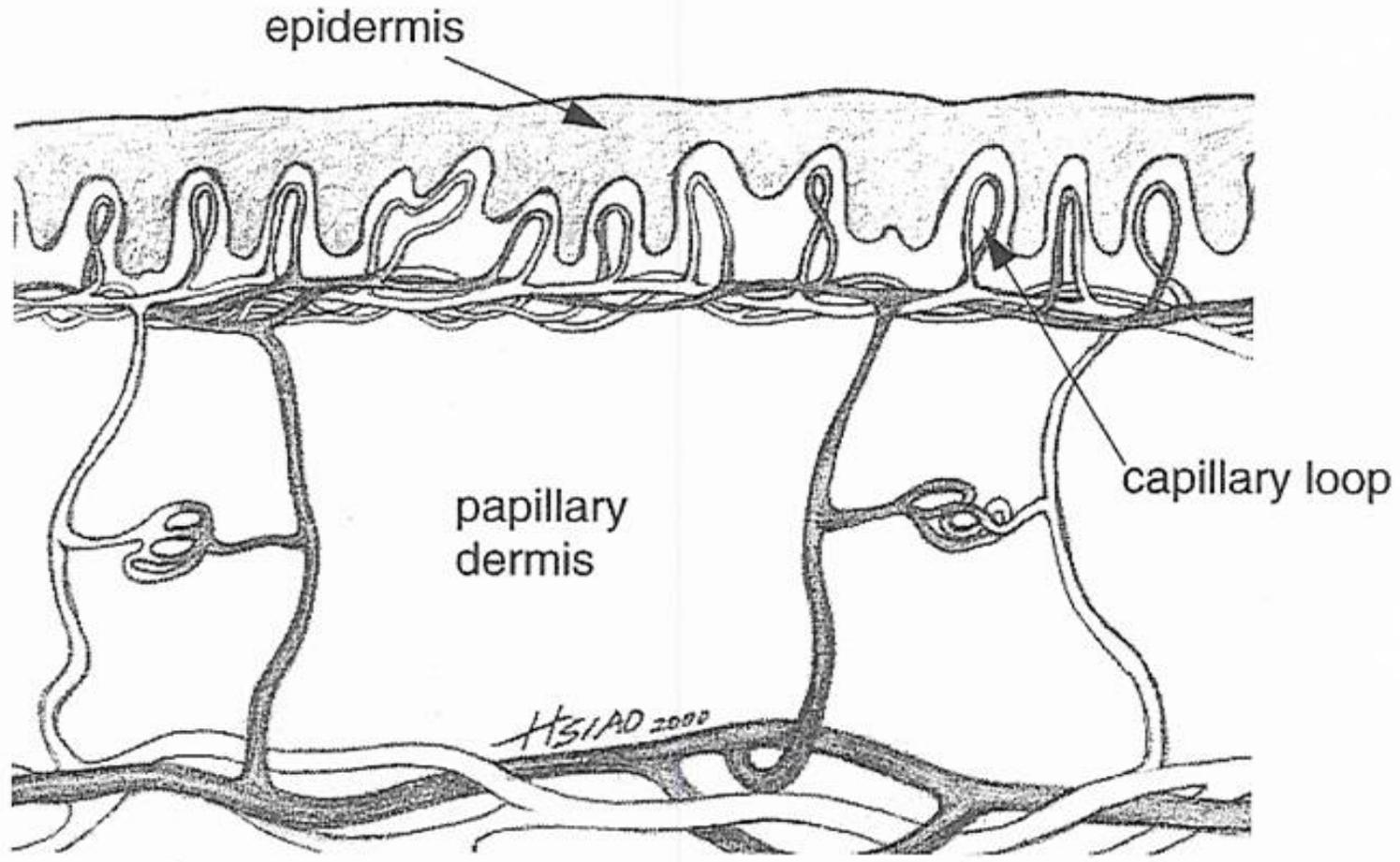
Simultaneous synthesis of epidermis and dermis.

- Uncultured KC seeded into DRT and grafted onto muscle substrate.**
- Contraction arrested and defect perimeter increased.**
- New tissue inside perimeter analyzed for skin (no hair).**

Evidence for partial synthesis of skin. Table 5.2

Courtesy of National Academy of Sciences, U.S.A. Used with permission.
© 2000 National Academy of Sciences, U.S.A. Source: Yannas, IV. "Synthesis of Organs: *In vitro* or *in vivo*?" *PNAS* 97, no. 17 (August 15, 2000): 9354-9356

**rete ridges with
capillary loops
and vascular plexus
underneath
(normal skin)**



Verify basement membrane. I: Immunostaining: Factor VIII for capillary loops

Histology image removed due to copyright restrictions.

75 μm

Verify basement membrane. II.

Immunostaining: $\alpha_6\beta_4$ Integrin for hemidesmosomes

Histology image removed due to copyright restrictions.
See Fig. 12 in Compton, C. C., et al. "Organized Skin Structure Is Regenerated In Vivo from Collagen-GAG Matrices Seeded with Autologous Keratinocytes." *Journal of Investigative Dermatology* 110 (1998): 908-916.
<http://dx.doi.org/doi:10.1046/j.1523-1747.1998.00200.x>

100 μ m


Compton et al., 2000

Verify basement membrane. III.

Immunostaining: Collagen VII for anchoring fibrils

Histology image removed due to copyright restrictions.

150 μ m

Compton et al., 2000

4. Partial synthesis of skin [Cont.]

In vitro-to-in vivo synthetic routes

- “Composite graft”. KC- and FB-seeded DRT cultured in vitro and form epidermis, before grafting.**
- In another version, use synthetic polymeric mesh instead of DRT (“living dermal replacement”).**
- FB are cultured inside collagen gel, then KC are seeded, before grafting (“living skin equivalent”).**

In vitro or in vivo? Skin synthesis

Skin

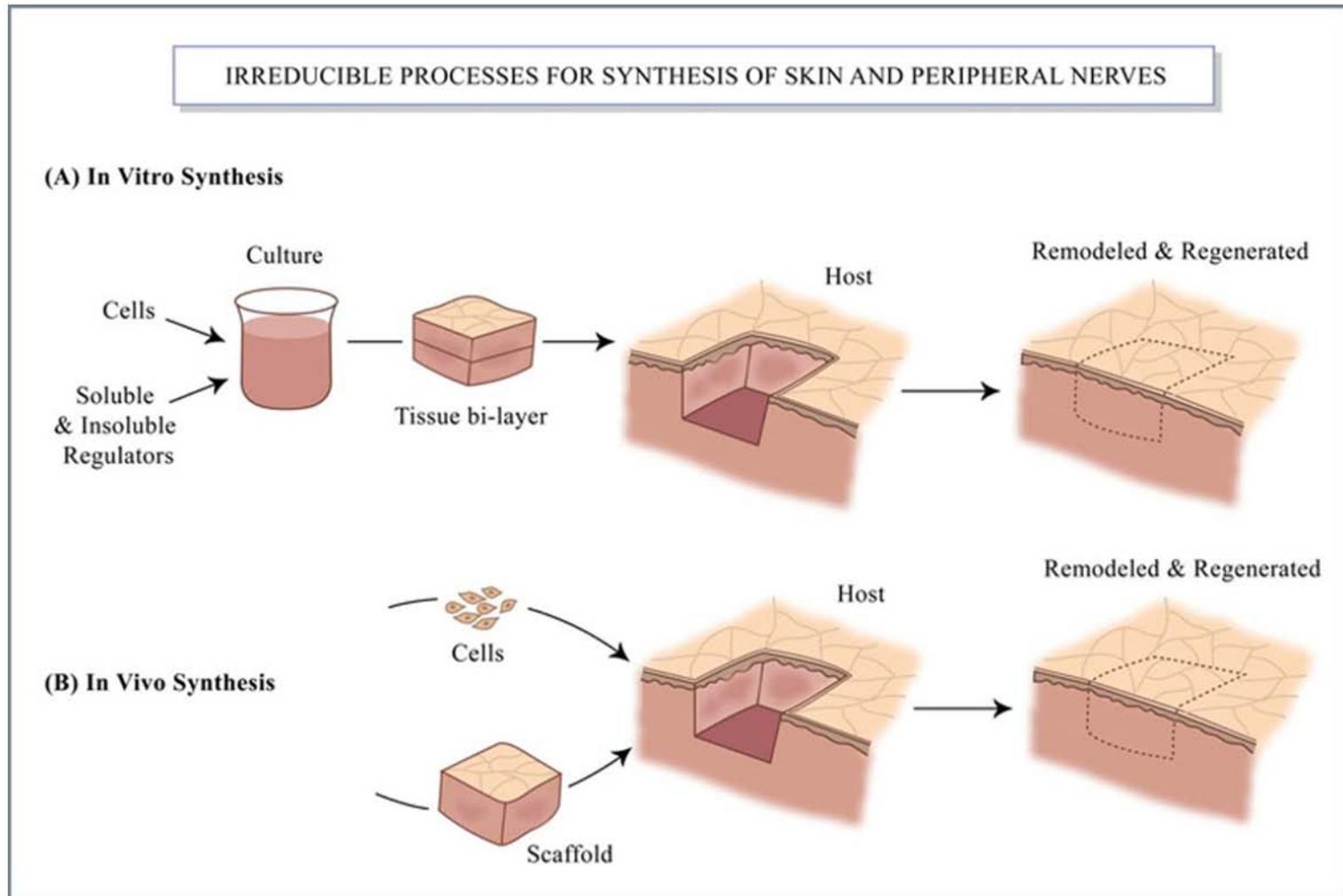


Figure by MIT OpenCourseWare.

5. Comparative regenerative ability of reactants

See Table 5.3.

Growth factors had no effect on final configuration (use defect closure rule).

Pharmacological agents, including steroids, had no effect.

KC sheets were ineffective.

Scaffolds, whether seeded or unseeded with cells (KC and/or FB), were very effective in suppressing contraction and scar synthesis, and inducing regeneration.

6. Overall mechanism of skin regeneration

How does wound contraction interact with skin regeneration?

- Is blocking of wound contraction necessary for regeneration?**
- Is contraction-blocking sufficient for regeneration? If not, what else is required?**
- Does regeneration occur independently of wound contraction or does contraction contribute to regeneration?**

Contraction-blocking vs regeneration

- Is contraction-blocking necessary?

Regeneration is not observed in presence of contraction blocking. However, need additional blocking protocols, e.g., specific pharmacological agents that block contraction, to prove cause and effect relation.

- Is contraction-blocking sufficient? No. In impaired wounds (e.g., diabetics), no contraction but also no regeneration observed.
- Does contraction of wound edges interfere with synthesis taking place inside wound edges? How do you separate contraction from synthesis?

Isolation of contraction from tissue synthesis during “island” grafting

Summary of experiment. DRT scaffold was seeded with keratinocytes and was grafted in the center of a skin wound, clearly away (by 1 cm) from contracting wound edges. The scaffold “island” was transformed in 14 days into a skin regenerate with epidermis and dermis. Wound contraction did take place, bringing wound edges close to, but not in contact with, island graft. Synthesis of new skin occurred without contact to contracting skin edges, demonstrating the independence of the regenerative process from wound contraction.

STUDY SKIN SYNTHESIS SEPARATELY FROM CONTRACTION OF WOUND EDGES.

Skin synthesis in “island” graft. Graft is separated by 1 cm from contracting wound edges.

LEFT: wound edge. RIGHT: left half of island graft. Island graft was prepared by grafting a collagen/GAG scaffold seeded with keratinocytes. Island graft was clearly separated from wound edges

Image removed due to copyright restrictions.

edge of wound

open wound undergoing contraction

island graft synthesizes skin

Keratinocyte cysts form in island grafts that are excessively seeded

500,000 cells/cm²

no keratinocyte cysts

1,000,000 cells/cm²

few keratinocyte cysts

3,000,000 cells/cm²

many keratinocyte cysts

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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 each of the regenerating organs (Dagalakis et al., 1980; Chang et al., 1990; Hsu et al., 2000).

Hypothesis: Regeneration leads to physiological stroma when it proceeds on the surface of a matrix that is a topological 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---about 14 days (Yannas et al., 1979; Yannas and Burke, 1980).

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

Isomorphous and synchronized tissue replacement rule

Similarity of configuration + synchronization of degradation rate of template → Rule of isomorphous and synchronized tissue replacement.

Rule: 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.

OVERALL MECHANISM OF SKIN SYNTHESIS

Fact: Contraction blocking is required but does not suffice to induce regeneration. This leads to a theory: Regeneration in adult = Contraction blocking + Tissue synthesis.

Other data suggest:

Tissue synthesis = Isomorphous and synchronized replacement of scaffold by new tissue

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