

III. Design case study: device for partial regeneration of skin. (outline of topics)

- 1. The clinical problem.**
- 2. Design at the patient scale.**
- 3. ... at the organ scale.**
- 4. ... at the cell scale.**

Text: TOR, Chs. 6, 7, 10.

1. The **clinical** problem.

- **Brief review of skin anatomy and physiology.** Epidermis, the moisture seal and bacteria seal. Dermis, the mechanical barrier and vital supporter of the epidermis. Skin receives from and sends signals to the environment.
- **Pathology (mortality and morbidity).** Loss of skin due to injury or chronic disease. Burns. Abrasion. Ulcers. Threats to life: Dehydration, sepsis. Threats to quality of life: Scarring and contracture.

1. The clinical problem. (cont.)

Patient population (narrow down to burn patients for better clinical focus; omit plastic surgery patients and ulcers patients).

Categories: First-, second- and third-degree burns.

The epidermis regenerates spontaneously provided there is a dermal substrate. The dermis does not regenerate spontaneously. Narrow down to third-degree burns.

1. The clinical problem. (cont.)

Approaches to solving problem. Allograft: Cadaver skin. Must immunosuppress patient, thereby complicating problem of survival. Epidermis is much more immunogenic than dermis. Use acellular dermis with some success. Xenograft: pig skin. Problem of bacterial and viral “load” of graft. Temporary dressings: synthetic polymeric scaffold seeded with keratinocytes and fibroblasts. Must be removed after a few days. No permanent cover. Gold standard: Autograft. Problems: not enough when burned area is >50%; patient traumatized (“donor site”).

Clinical scale: bottom line

¶ The device will be directed toward patients with third degree burns.

¶ The gold standard is the autograft.

2. Design at the **patient** scale.

- a. The burn patient population: Large areas , scale about 1 cm², of missing skin.
- b. Short-term care problem (<20 days): Survival inside clinic.
 - Massive bacterial invasion. Risk: lethal sepsis.
 - Massive dehydration. Risk: lethal shock.
 - Loss of thermoregulation. Risk: acute discomfort.

2. Design at the **patient** scale. (cont.)

c. Long-term care problem (>20 days):

Quality of life outside clinic.

- **Contraction.** Prevents movement if occurring near joints.
- **Scar.** Mechanically weak cover, reduced tactility, cosmetically objectionable. No thermoregulation.

¶ Therefore, the device must provide both temporary and lifelong physiological cover to patient.

3. Design at the **organ scale**

- a. Anatomy of the missing skin. Epidermis, dermal-epidermal junction (DEJ), dermis, accessory organs.**
- b. The epidermis keeps water in, bacteria out.**
- c. The epidermis bonds to the dermis (DEJ).**
- d. The dermis protects the epidermis mechanically and metabolically. Nerve endings inside the dermis provide sense of touch (tactility).**

**The epidermis is
spontaneously
regenerative**

Diagram removed due to copyright restrictions.

3. Design at the **organ scale (cont.)**

- e. Accessory organs located inside the dermis (sweat glands, hair follicles) provide thermoregulation.**
 - f. The epidermis regenerates spontaneously provided there is dermal substrate.**
 - g. The dermis of the adult mammal does not regenerate.**
- ¶ Therefore, the device need only regenerate the dermis. The epidermis will then take care of itself.**

4. Design at the **cell** scale

- a. A full-thickness skin wound closes spontaneously in about 20 days by two processes. None of these regenerates the dermis:
- Contraction of wound edges
 - Synthesis of scar (epithelialized)
- b. The device must block these two spontaneous processes for wound closure. Basic concept: If contraction is blocked, wound closure will occur by regeneration (hypothesis; see basic data).

4. Design at the **cell** scale. (cont.)

- c. Contraction of wound edges is caused, in part, by mechanical forces generated by myofibroblasts. These cells form dense networks which pull the wound edges toward center of wound. The same cells probably also synthesize scar.
 - d. The device must inhibit formation of dense networks of myofibroblasts (MFB) inside the wound. Solution: Prevent cell-cell bonds, which form cell network, by extensive formation of cell-device bonds.
- ¶ Block contraction, induce dermis regeneration.

Design requirements: Bottom-line summary

- ¶ The device will be directed toward patients with third degree burns.**
- ¶ The gold standard is the autograft.**
- ¶ The device must provide both temporary and lifelong physiological cover to patient.**
- ¶ It need only regenerate the dermis. The epidermis will then take care of itself.**
- ¶ Block contraction, induce dermis regeneration.**

Parenthesis: Look at basic research data: they show that contraction inhibits regeneration, and that certain scaffolds can block contraction

1. Skin wounds.

2. Peripheral nerve wounds.

1. Skin wounds.

Wound contraction is incompatible with regeneration

- **Extensive data suggest that, during healing of severe injury, contraction antagonizes regeneration (see Text, Ch. 8.).**
- **Therefore, attempt regeneration using devices that block contraction (without blocking other aspects of healing process.)**

Contraction of standard skin wound

Images removed due to copyright restrictions.

Template blocks contraction in skin wound

no template

contractile cells

Images removed due to copyright restrictions.

with template

contractile cells

Structural features of ECM analogs used as tube fillings in skin and nerve regeneration

Images removed due to copyright restrictions.

2. macromolecular structure (ligand duration)

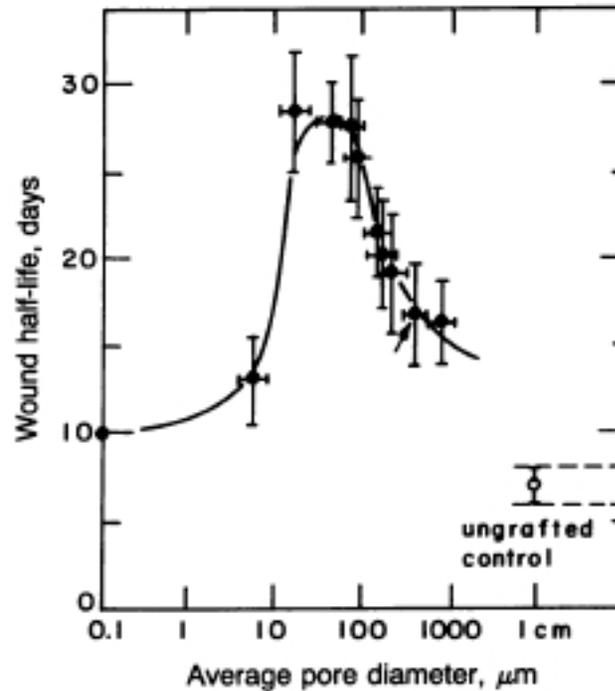
3. chemical composition (ligand identity)

4. orientation of pore channel axes

Dermis regeneration template

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100 μm



Contraction inhibited maximally in scaffold pore diameter range 20 μm — 120 μm

FIG. 1. Variation of the skin wound half-life (the time required for a wound to contract to 50% of the original area) with the average pore diameter of CG copolymer used as the graft. The arrow marks the average pore diameter of the copolymer series used to obtain the data in Fig. 2.

Courtesy of National Academy of Sciences, U.S.A. Used with permission.

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Source: Yannas, I., et al. "Synthesis and Characterization of a Model Extracellular Matrix that Induces Partial Regeneration of Adult Mammalian Skin." *PNAS* 86 (1989): 933-937.

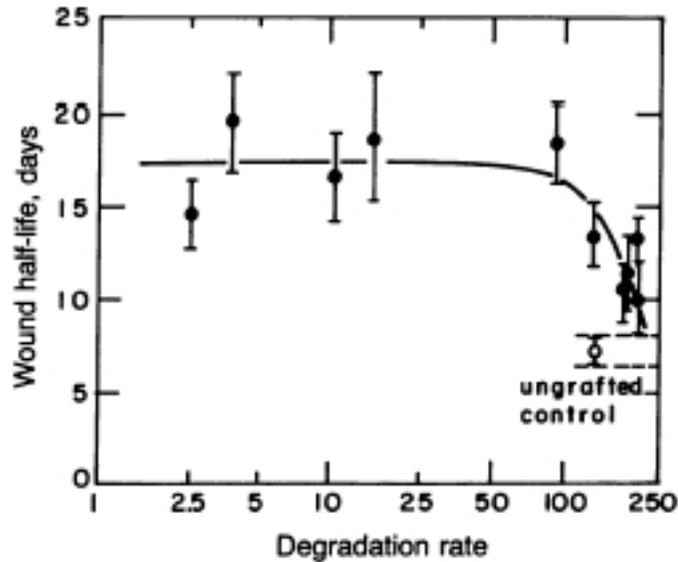


FIG. 2. Variation of the skin wound half-life with degradation rate R of CG copolymer used as the graft. The degradation rate is in empirical units, which are defined in the text.

Contraction of skin defects inhibited maximally in scaffold degradation rate range about 2 — 110 enz units (half-life 10-15 d)

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Source: Yannas, I., et al. "Synthesis and Characterization of a Model Extracellular Matrix that Induces Partial Regeneration of Adult Mammalian Skin." *PNAS* 86 (1989): 933-937.

Kinetics of defect area closure using three protocols

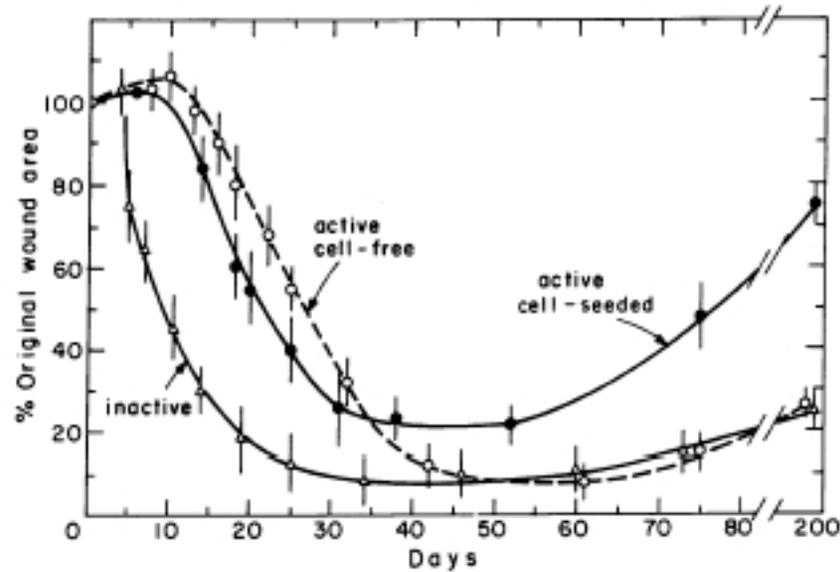


FIG. 3. Change in the original wound area with time observed when full-thickness guinea pig skin wounds were grafted with inactive CG copolymers (Δ), active cell-free CG copolymers (\circ), and active cell-seeded CG copolymers (\bullet).

Courtesy of National Academy of Sciences, U.S.A. Used with permission.

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Source: Yannas, I., et al. "Synthesis and Characterization of a Model Extracellular Matrix that Induces Partial Regeneration of Adult Mammalian Skin." *PNAS* 86 (1989): 933-937.

4. Design at the **cell scale. (cont.)**

e. After blocking contraction and scar synthesis the device must be degraded to allow regeneration, leaving nontoxic debris in its place.

¶ Extensive MFB-device bonds must be formed temporarily.

Visualization of device.

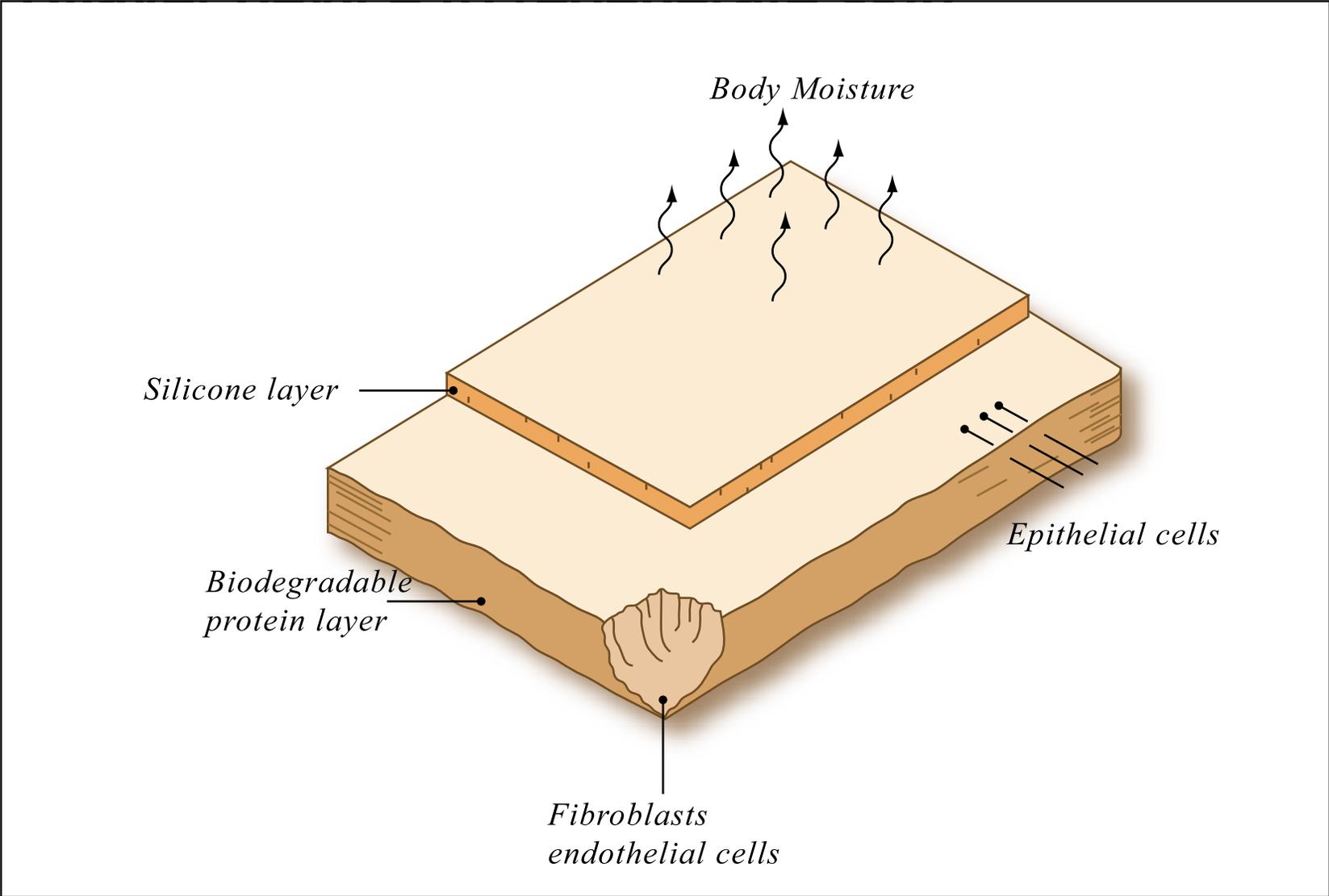


Figure by MIT OCW.

Visualization of the device

A two-layer device.

- **Short-term care: The top (distal) layer replaces the function of the missing organ.**
- **Long-term care: while the bottom (proximal) layer regenerates the dermis. The epidermis will then form spontaneously over the new dermis.**

Review of current conception of the device

- ¶ Provide burn patient with regenerated dermis and epidermis over lifetime.**
- ¶ Neglect large (>5 cm) wounds.**
- ¶ Neglect absence of thermoregulation (sweat glands and hair follicles) by new skin.**

Summary of design criteria for severely burned patient population

- **Patient scale:** The device must provide both temporary and lifelong physiological cover.
- **Organ scale:** Must regenerate the dermis only. The epidermis will then take care of itself.
- **Cell scale:** Regenerate by blocking contraction. Use scaffold that bonds extensively with contractile cells over critical period.

Conception of the device (cont.)

- **Distal layer**: Functions short-term at the organ scale. A suturable barrier to bacterial invasion and dehydration. Removed on Day 20 in the clinic.
- **Proximal layer**: Functions long-term at the cell scale. A molecular scaffold ("regeneration template") captures MFB cells and thus blocks wound contraction and scar synthesis. It induces synthesis of dermis and eventually, it is degraded by wound enzymes. The epidermis regenerates spontaneously over the new dermis at 1 mm/day: Design neglects wounds larger than 5 cm; also, neglects thermoregulation.

Organ scale. Detailed functional design of distal layer

- a. Mechanically affix device to open wound (organ scale): Surgical handling and suturability of distal layer. Tensile strength. Young's modulus. Thickness.
- b. Control of moisture flux to physiological level (organ scale): Permeability.

Detailed functional design of proximal layer (cell scale)

- a. Physicochemical adhesion to open wound (organ scale): Eliminate air pockets between device and wound ("dead space") thereby forming continuous liquid phase for diffusion of molecules (eg., growth factors) and migration of cells from wound to device. Surface chemistry (wetting of wound bed) and porosity (capillary forces bonding device to wound bed) of proximal layer.

Detailed functional design of proximal layer (cell scale)(cont.)

- b. Cell migration into proximal layer: Critical cell path length. $S = rL^2/DC_0 =$ rate of consumption of critical nutrient by cell/diffusion of nutrient from wound bed. At $S = O(1)$, identify the critical cell path length, $L_c = O(100 \mu\text{m})$, which defines the maximum thickness of the proximal layer. Cells can migrate into device only if average pore diameter of proximal layer is $d_{\text{ave}} > 10 \mu\text{m}$.

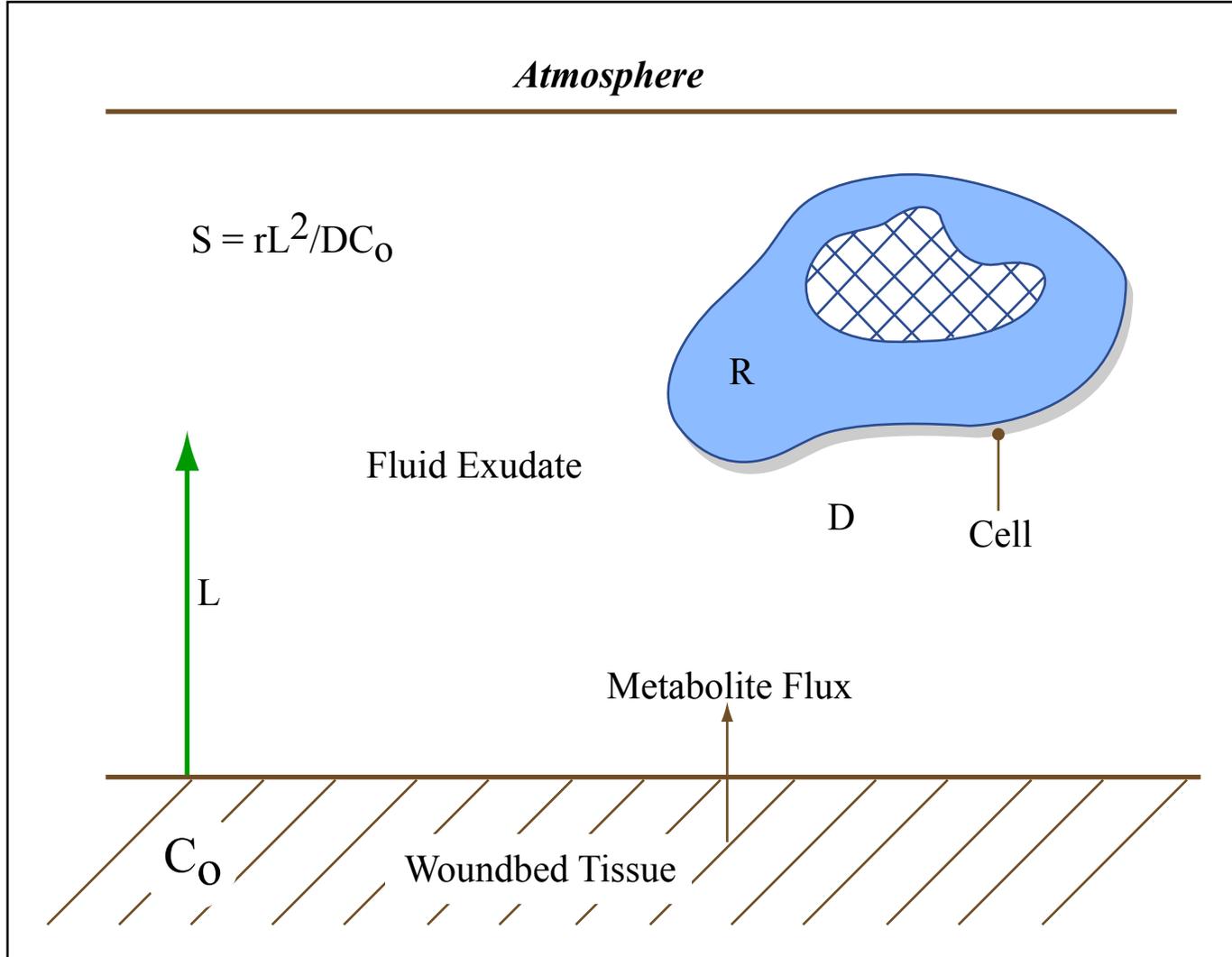


Figure by MIT OCW.

Stepwise modeling of process of regeneration

- What distance can host cell stray away from host tissue into device? Balance metabolite diffusion from host to cell with its consumption inside cell. Critical cell path length, L_c , controlled by value of S :

$$S = rL^2/DC_o$$

where: r rate of consumption of critical cell metabolite; L distance from host metabolite reservoir; D metabolite diffusivity; C_o concentration of metabolite in reservoir

At $S = O(1)$, L_c is of order $100 \mu\text{m}$.

Stepwise modeling...(cont.)

- What are upper and lower bounds of template pore diameter, d ?

Lower bound set by minimum pore size through which cell can migrate inside scaffold. Upper bound set by requirement for high enough specific surface (low enough pore diameter) for sufficiently high density of cell-matrix (receptor-ligand) interactions to occur.

For dermis, data show $20 \mu\text{m} < d_{\text{ave}} < 120 \mu\text{m}$.

Stepwise modeling...(cont.)

- How long should template remain in undegraded state? What is the relation between rate of biodegradation and rate of synthesis of new tissue?

Data show $t_b/t_h = O(1)$

where t_b is the time constant for biodegradation of template and t_h is the time constant for healing.

Steady state occurs when the time constants are roughly equal. Corresponds to

“ isomorphous tissue replacement”.

¶ Template should remain undegraded for about 2 weeks.

Detailed functional design of proximal layer (cell scale) (cont.)

- c. Specific cell-matrix interaction inside the proximal layer: Leads to blocking of contraction and scar synthesis. Requires large specific surface of ECM analog for porous proximal layer. Pore diameter $d_{ave} < 120 \mu\text{m}$ to provide sufficiently large specific surface for cell-matrix interaction. GAG/collagen w/w $> 1/99$ to provide sufficient density of cell binding regions.

Detailed functional design of proximal layer (cell scale) (cont.)

d. Residence time of device inside wound. Cell device bonds must be maintained over entire healing period, $t_h \sim 20$ days. Device must biodegrade not much later than 20 days, $t_b \sim 20$ days.

Detailed functional design of proximal layer (cell scale) (cont.)

"Isomorphous tissue replacement":

Regenerated tissue is synthesized at a rate which is of the same order as the rate of degradation of the proximal layer. $t_b/t_h = O(1)$. GAG slows down degradation of collagen by wound enzymes. Additional slowing down of degradation of GAG/collagen complex by chemical cross-linking to produce network with $M_c = 12,000 \pm 5,000$. At $t_b \gg 20$ days, scar forms around device. At $t_b \ll 20$ days, device has no effect on contraction or scar synthesis.

Clinical trial of bilayer design

Clinical trial protocol

- 1. Graft the bilayer on burn patients.**
- 2. After 20 days, replace silicone layer with epidermal autograft.**

KINETICS OF SKIN SYNTHESIS I.

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**Scaffold
seeded
with epithelial
cells**

**Scaffold
slowly
degrading**

**Butler et al.,
1998**

KINETICS OF SKIN SYNTHESIS II.

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**Scaffold
degraded;
diffuses
away**

Butler et al.,
1998

Verify basement membrane. I: Immunostaining with Factor VIII to detect capillary loops

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75 μm

Verify basement membrane. II.

Immunostaining: $\alpha_6\beta_4$ Integrin for hemidesmosomes

Photo removed due to copyright restrictions.

100 μ m



Compton et al., 2000

Verify basement membrane. III.

Immunostaining: Collagen VII for anchoring fibrils

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150 μ m



Compton et al., 2000

6-yr-old burn victim – total skin loss on upper and lower abdomen

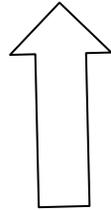
patients of Dr. John Burke

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**autografted area on
upper abdomen**

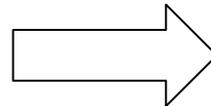


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grafted template

6 years later



Case 1

- Patient presented in the emergency room with massive burns in the face.
- Burn surgeon excised burned skin (debriding), thereby deliberately **generating a deep wound** all over the face.
- **A scaffold was then applied** to the wound.
- Slides from Matt Klein, MD, University of Washington, Seattle, WA (2005)

Summary of treatment

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Pre-Excision

Postoperative day 28

Case 2

- Patient suffered trauma to the skin following exposure to alkali. These wounds were allowed to heal by contraction and scar formation.
- Surgeon **generated new deep wounds** by deliberate excision of the scars.
- A **scaffold was then applied** to the open wounds.
- Slides from Dr. Andrew Byrd, Bristol, UK (2002).

Photos removed due to copyright restrictions.

Case 3

- A 79-year-old woman presented with a chronic skin ulcer in the foot.
- The surgeon excised the ulcerous wound bed and **generated a fresh wound.**
- **A scaffold was then applied** to the fresh wound.
- Data from clinical trial in Phoenix, AZ by M.E. Gottlieb, MD and J. Furman, MD. Published in J Burns & Surg Wound Care 2004;3(1):4. Available from: URL: <http://www.journalofburns.com>.

Example of induced regeneration in a chronic skin wound. “A 79 year old woman presented with an ankle ulcer of several years duration. ...It was excised, including bone fragments and the arthrosis, and (a biologically active scaffold) was used to close the wound and the structures underneath. Seen here 11 days later, periwound inflammation is gone. The wound healed and has remained so, seen here a year later.” (*underlining supplied*)

Photos removed due to copyright restrictions.

Chronic wound

Post-excision

11 days after grafting

Gottlieb and Furman, 2004