Skin Regeneration

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Disclosures:
none.

Content available: www.nebraskasurgicalresearch.com
Overview

• Clinical problem
• Established treatments
• Research approaches
• Our strategy
Skin Loss: Clinical Problem

• Normal anatomy
• Healing v. Regeneration
• Sequelae of skin loss
Normal skin anatomy (rat)
Normal skin anatomy

**structural components**
- epidermis
- basal lamina
- **dermis**
- blood vessels
- hair follicles
- glands
- cutis (nail, claw, horn)
- (subcutaneous adipose)
- (skeletal muscle)

**functions**
- barrier
- temperature regulation
- sensation
- coloration
- immune response
- synthetic
- specialized
Healing vs. Regeneration

Excisional wound: natural history

Dorsum of rat: 2 x 2 cm full-thickness excision, followed for 204 days
Growth

(RJ Goss, 1992)
Growth Redux

intact organism → pathologic process → tissue loss

wound healing → compensatory hypertrophy

EPIMORPHIC REGENERATION
Healing v. Regeneration

Amphibian limb amputation

Figure 2-4. Montage of individual newt limbs amputated across lower or upper arms, as photographed after 7, 21, 25, 28, 32, 42, and 70 days of regeneration (From Goss R: Principles of Regeneration. New York, Academic Press, 1969.)
A type of growth: wound healing (corneal injury)
Regeneration vs. conventional healing

Figure 3. Repair of dermal injury is analogous to reconstruction of a picket fence postinjury.
Elastin: scar vs. dermis
Negative growth:
atrophy
(skeletal muscle denervation)

rat tibialis anterior
(anti-laminin)

(Anat Rec 2001;264:203)
Regeneration through the phyla

The larger and/or more advanced the animal, the less it can regenerate.
Tissue-Specific Mammalian Regenerative Capacity

HIGH
1. Epidermis
2. Liver (hyperplasia)
3. Endothelium
4. Epithelium

LOW (but theoretically possible)
1. Everything else

Holy Grail
So what is the problem with healing?

In most cases, nothing. But...
Sequela of skin loss:

Burn wound contracture
Abnormal healing response:
Keloid scarring
“Tissue repair is designed to reconstruct morphological integrity; epimorphic regeneration is designed to restore function.”

“Animals can live without epimorphic regeneration but not without tissue repair.”

Richard J. Goss, 1992

Wound healing = quick fix; poor function

Regeneration = complicated; normal function
Skin Loss:
Established Treatments

- Skin flaps/grafts
- Epidermal substitutes
- Dermal substitutes
- Combined (epiderm + derm)
Skin flaps: simple

Excisional wound (rat dorsum)
Free flap
(treatment of contracture)
Skin grafting

Harvest
(300-400 μm thick)

Forearm graft
Skin grafting:

full- vs. split-thickness
Skin grafting: split-thickness
Skin grafting:

“Gold Standard” for skin replacement

Disadvantages

- Limited source of material
- Wound contraction
- Cosmesis
- Donor site morbidity
Skin grafting:
Donor site morbidity
Skin loss treatments: Epidermal substitutes

- **Strategy:** cultured autologous keratinocytes/hair follicle cells (CEA)

- **Examples:** Epicel®, Laserskin®, Epidex™, MySkin™
Epidermal replacement
Epidermal substitutes: Autologous keratinocytes

Advantages:
• No rejection
• No large donor sites

Disadvantages:
• Fragile, no supportive dermis
• Time requirement (culture), no storage
Skin loss treatments: Dermal substitutes

- Strategy: natural, semisynthetic, or synthetic matrix, ± fibroblasts

- Examples: Alloderm®, Biobrane®, Dermagraft®, Integra®, Permacol™, Transcyte®
Dermal replacement
Dermal substitute: Alloderm®

Composition: processed cadaveric human dermis

Advantages
• Minimal rejection
• Simplicity, tractability

Disadvantages
• No cellular component
• Expensive
Dermal substitute: Biobrane®

Composition: nylon mesh + silicone membrane + porcine ECM

Advantages
- Simplicity
- Large area coverage

Disadvantages
- Not a permanent replacement
Dermal substitute: Dermagraft®

Composition: allogeneic neonatal fibroblasts in polyglactin mesh

Advantages
- Minimal rejection
- Absorbable ECM

Disadvantages
- Complexity
- Small area coverage
Dermal substitute: Integra®

Composition: bovine col + GAGs topped with silicone

Advantages
• Encourage cellular ingrowth
• Integrates with host

Disadvantages
• Needs autograft after silicone removal
• Bovine allergy risk
Dermal substitute: Permacol™

Composition: treated porcine dermal collagen

Advantages
• Non-immunogenic
• Supports ingrowth from host

Disadvantages
• Needs autograft after incorporation
• Expensive
Dermal substitute: Transcyte®

Composition: collagen-coated nylon seeded with allogeneic neonatal fibroblasts, topped with nylon

Advantages
• Integrates with host, encourages ingrowth

Disadvantages
• Nonabsorbable
• Not permanent
Skin loss treatments:
Combined epidermal/dermal substitutes (composites)

- Strategy: cultured allogeneic cells populating a bilaminar structure

- Examples: Apligraf®, Orcel®
Composite substitute: Apligraf®; Orcel®

**Composition:** allogeneic neonatal keratinocytes + fibroblasts, type I col matrix with cytokines

**Advantages**
- Minimal rejection
- Rational design

**Disadvantages**
- Complexity, time, expense
- Limited area coverage
Skin Regeneration: Research Approaches

- Endogenous regeneration
- Fetal paradigm
- Tissue engineering
Research approaches:
Endogenous regeneration

- Enable inherent regenerative mechanism (“inside-out” approach)
- Requires understanding of regeneration vs. healing
- Resident stem cell biology relevant
“Mammals do not regenerate”

Exceptions:

• Antler growth

• Rabbit ear regeneration

• Distal fingertip amputations in mice and young children

(Others?)
Dedifferentiation in nature?

Blastema formation:
central event of limb regeneration

Figure 2–5. Schematic representation of how a vertebrate appendage regenerates. Following amputation (A), the epidermis migrates across the severed mesodermal tissues (B). The cells of the latter dedifferentiate distally (circles), and migrate into the blastema where they proliferate (+) (C). As the blastema elongates, its more proximal cells redifferentiate while a zone of proliferation persists distally (D). Regeneration ceases when all of the blastema cells have differentiated (E).
Dedifferentiation in nature?

Limb amputation blastema

What’s in there?

A. Stem cells?
B. Dediff cells?
C. A & B?
Stem Cells: Properties

1. Self-renewal (can make a copy of itself)
2. Differentiation (can become another cell type)
3. Can reconstitute tissue *in vivo* [informal]
Stem Cell Primer

- ESC: embryonic stem cell (blastocyst ICM)
- iPS: induced pluripotent stem cell
- Tissue stem cell (adult or somatic stem cell)
- MMSC: multipotent mesenchymal stromal cell
Stem cell potency

Totipotent
- zygote
  - Mesoderm
  - Endoderm
  - Ectoderm
  and
  - Germ
  - Trophoblast
  (i.e., *entire* organism)

Pluripotent
- embryonic stem
  - Mesoderm
  - Endoderm
  - Ectoderm

Multipotent
- hematopoietic stem
  - Organ specific

Unipotent/Progenitor
- intestinal crypt
  - Cell specific

PLASTICITY
Stem cells & dedifferentiation

undifferentiated stem cell

forward pathway: canonical (differentiation)

reverse pathway = DEDIFFERENTIATION
- Does this pathway exist?
- If so, where, how, and how frequently?

differentiated daughter cell
Research approaches: Fetal paradigm

- Mammalian fetus: regenerative response instead of inflammation/healing
- Transition point *in utero*, after which regeneration no longer occurs
- Phenomenology followed by mechanistic studies (no applications yet)
Scarless healing in the dermis of the mammalian fetus

Excisional wounds in the fetal rat after 72 hr

(Plast Reconstr Surg 2001;160:209)
Scarless fetal healing (cont’d):
collagen organization in the E16 fetus

72 hr after wounding on E16  unwounded
Scarless fetal healing (cont’d):
collagen organization in the **E18** fetus

72 hr after wounding on E18  unwounded
Fetal paradigm:
Comparison with adult

• Fetal ECM: enriched in type III collagen, hyaluronic acid, tenascin-C

• Elevated levels of TGF-β3, IL-10; decreased TGF-β1/2, IL-8, many others

• Differences in fibroblast phenotype
Research approaches:
Tissue engineering

• Replace lost tissue with engineered construct ("outside-in" approach)

• Requires understanding of how implants interact with host

• Pluripotent stem cell biology relevant
Tissue engineering: scaffolds for construction

• Materials include organic polymers (e.g., polyglactin, polylactide), gelatin, chitosan, PEG hydrogels

• A variety of synthetic techniques can control micro- and nano-architecture (e.g., fiber diameter, pore size, physical properties)
Tissue engineering: “Smart” biomaterials

- Older strategy of 100% synthetic matrix failed
- Newer strategy uses semisynthetic materials (e.g., polymer coated with ECM)
- Coating encourages cellular ingrowth
Tissue engineering: ECM coating

- Adsorbed or covalently bound to scaffold
- Examples include: type I/III collagens, elastin, HA, fibronectin, RGD peptides, other peptide mimetics
Tissue engineering: cellular embedding

- Embed vs. ingrowth (chemoattraction)
- Cell type: differentiated vs. stem
- If stem, what type (MSC, iPSC, ESC, etc.)
Skin Regeneration: Our Strategy

- Nanoengineered scaffold
- ECM surfacing
- Cells (MSC + fibroblast)
- Cytokine slow-release
- Multi-layer recapitulation
Objective:

To develop a replacement therapy for epidermis/dermis (i.e., to engineer a complete skin equivalent for clinical use)
dermal replacement: strategy

• Material can be manipulated at the nano-level for architectural and physical properties

• Material can be engineered to contain nanoparticles for slow-release of various cytokines/growth factors
collagen matrix with cells:
- dermal fibroblasts
- mesenchymal “stem”

“neodermis”

dermal replacement: strategy
dermal replacement: 
strategy

basal lamina 
(type IV collagen, laminin)

neodermis
dermal replacement:
strategy

keratinocytes
(epidermis)
dermal replacement: strategy

finished skin equivalent

plug into animal model
Aerosol Delivery System

[to go movie file “ADS.mov”]
“Regeneration” Assay

(wound contraction)

Additional endpoints
- Tensile strength
- Microscopic morphology
Looks easy, but ... potential problems:

- Co-culture conditions
- Blood supply to implant
- Infection (see next item)
- Inflammation at interface
- Strength & durability
- Nerves, glands, hair, etc.
- Translation from rodents to humans
- Cellular source
Engineered tissue will require an immediate blood supply

(Donor kidney just prior to transplantation)
...Engineered tissue may need to function immediately after implantation.
Inflammation

(Excisional wound bed, postwounding day 3)
Conclusions

1. Proofs of concept are readily available, but...

2. Translation into practical treatments have been rare

3. Road to practical treatments will be long and difficult
Take-home message:

Healing bad, regeneration good.

Current reality:

“When my liver fails, don't give me a bone marrow transplant [i.e., stem cells], give me a liver.”

Irving Weissman, 2004