Tissue Healing by Regeneration and Repair with Fibrosis

Tibor Krenacs

Medical Students 2015/16
Tissue integrity – essential for life

INJURY

- Neutralization of toxic agents
- Elimination of toxic agents + tissue debris

INFLAMMATION

- Restoration of tissue structure and function (Damage: caused by trauma and/or toxic agents + inflammation)

REGENERATION

- Tissue repair when reticular fibers & basement membranes (ECM) damaged (Cause: Serious injury, chronic inflammation)

FIBROSION

Fibrotic scarring

- e.g. myocardial infarction „ischemic hearth disease“ huge burden public health 7.4 million death/2012

Inflammation: serious injury; innate + adaptive immunity, proteases, fibrosis

Regeneration

Inflammatory cells, kemokines, cytokines, growth factors, proteases
Amphibians e.g. salamandra regenerate extremities

Blastema cells – undifferentiated stock of stem cells (proximal neurons + Schwann cells)
Embryonal wound healing – without scarring

Day-18th chicken embryo – wing bud incision wound

Similar in human embryo before the 3rd trimester

- TGFβ3 > TGF-β1-2; Reverses after birth
- Immature vs mature immune system

Regeneration – Repair with Fibrosis

- cascades of overlapping events
- interactions between cell-cell and cell-matrix

Skin wound as a model

- **Bleeding - Coagulation:** Isolation from the environment
- **Inflammation:** Prevention of infection/septicemia
- **Debridement:** Elimination of tissue debris
- **Migration, proliferation:** Replenishment of lost tissue
- **Epithelialization:** Parenchyma regeneration
- **Angiogenesis:** Nutrition of granulation tissue
- **Fibroplasia:** Fibroblast invasion, matrix production
- **Remodelling:** Generation and degeneration of ECM
- **Contraction:** Close up wound edges
- **Resolution:** Restoration of appearance and function
**HEALING:** Aiming to regenerate tissue structure and function

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| • Signaling pathways | • **Regeneration:** *Rexitutio ad integrum*

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**Repair of specific tissues**

**Pathology of tissue repair**
Cell proliferation – Cell cycle

- **Promoters** - Cyclin-cdk complexes

  → Rb phosphorylation

- **E2F**

- **Inhibitors** - Cyclin dependent kinase inhibitors

2015 Nobel price in chemistry
T. Lindahl
P. Modrich
A. Sancar

DNA REPAIR

Chromosome duplication

Check for DNA damage (G_{1}/S checkpoint)

Restriction point
Centrosome duplication
Growth in mass

G_{1}

Licensing

M

Mitosis

G_{2}

Check for damaged or unduplicated DNA (G_{2}/M checkpoint)

G_{0}

Cell division

Growth factors

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Regulation of baseline cell population

Committed normal cell population

- BALANCE
- apoptosis
- cell death

INJURY!
- elevated cell decay

STEM CELLS

Cells able to regenerate throughout life
- self-renewal
- asymmetric cell division

Intermitotic cells
- reversibly postmitotic cells

proliferation

1. symmetric

2. asymmetric

A B C
STEM CELLS

Embryonic stem cell  *Form different tissues*
**Pluripotent**: inner cell mass cells of morula (ecto-, meso-, entoderm)

Adult stem cells
**Multipotent**: some cell types e.g. bone marrow (or mesenchymal) stem cells can form all subtypes of hemopoietic cells
**Unipotent**: skin, GI track epithelial stem cells

Maintain the compartment regeneration

In vitro fertilization

MHC - histocompatibility
Transplant rejection
Reprogramming - Induced pluripotent stem cells (iPS)

2012 Nobel prize in Physiology-Medicine: Sir John B. Gurdon and Shinya Yamanaka "for the discovery that mature cells can be reprogrammed to become pluripotent"

**Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors**

Kazutoshi Takahashi\(^1\) and Shinya Yamanaka\(^1,2,3\)

\(^1\) Department of Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan
\(^2\) CREST, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan

Cell 126, 663–676, August 25, 2006

The Developmental Capacity of Nuclei taken from Intestinal Epithelium Cells of Feeding Tadpoles

by J. B. Gurdon\(^1\)

From the Embryology Laboratory, Department of Zoology, Oxford


Reprogramming

Oct3/4, Sox-2, c-Myc, Klf4, Nanog

Therapeutic cloning
CD34+ stem cells

Potential uses of stem cells

- Stroke
- Traumatic brain injury
- Learning defects
- Alzheimer's disease
- Parkinson's disease
- Baldness
- Blindness
- Deafness
- Amyotrophic lateral sclerosis
- Myocardial infarction
- Muscular dystrophy
- Diabetes
- Multiple sites: Cancers

Missing teeth

Bone marrow transplantation (currently established)

Spinal cord injury

Osteoarthritis

Rheumatoid arthritis

Crohn's disease
Regeneration - proliferative capacity of parenchymal cells

*Labile cells/tissues:* *continuously replicating* – Intermitotic cells

>1.5% of the cells in mitosis, may be destroyed by sublethal injury

- hemopoiesis, skin, **oral mucosa**, GI tract, respiratory, urinary tract

**Stem cells** – multipotent/unipotent – **Efficient regeneration**

**Stem cell „niche” (microenvironment)**

![Ki67](image1)

![Intestine](image2)
Cornea regeneration after excimer laser ablation

Dr. Imola Ratkay-Traub MD PhD: FDA approval of femtosecond laser (LASIK)
(Prof. Tibor Juhasz, Univ. Michigan, Ann Arbor)
Cornea regeneration 24h post laser treatment

Proliferation – Ki67 protein

A. Control – Resting cornea

B. Limbal – under regeneration

C. 

D. 

Connexin direct cell-communication compartmental functions – cell network

E. 

F. 

G. 

H.
**Stabil cells/tissues:** terminally differentiated G0 phase cells (reversibly postmitotic) - adapt well, high metabolic activity, longevity - liver, kidney, pancreas, endometrium, endocrine glands - endothelial cells, fibroblasts, smooth muscle

**No stem cells** (except in liver), differentiated cells can proliferate

**Permanent cells/tissues:** terminally differentiated cells, **no proliferative capacity** - neurons of the central nervous system, myocardium - if injured repaired by connective tissue scar (skeletal muscle? – satellite cells)

Liver regeneration

Prometheus’s punishment by Zeus – eagle eating from his liver
Healing of special tissues

**Myocardium** (permanent cells): complex function, longevity, no regeneration
no stem cells, the same for endocardium; Healing with scar:
- arrhythmias
- damaged pumping function

Healing of special tissues

**Nervous system:** a neurons can not replicate (SVZ, hyppocampus dentate gyrus)

**Central**
- No axon regeneration
- Repair involves microglia, astrocyte proliferation (inflammation)

**Peripheral**
- Axon regeneration if broken ends aligned accurately; if not traumatic neuroma: fibrosis, abortive axon prolif.
Permanent cell/tissue, BUT satellite cells (stem cells)

If matrix (endo-, perimysium) preserved muscle fibers can fully regenerate (however, long cells, stroma is broken - fibrosis)
Skeletal muscle regeneration

Notexin (venom of the tiger snake)
Growth factor receptor subtypes

Protein kinase (growth factors)

G-protein coupled receptor (kemokines)

Without intrinsic kinase activity (cytokines)

Regeneration medicine

Blocking of inhibitory factors of retinal axon regeneration

PTEN → Akt/mTOR

SOCS3 → JAK/STAT3

2012 Nobel Prize in Chemistry
Robert J. Lefkowitz and Brian K. Kobilka
"for studies of G-protein-coupled receptors"

Growth factors in wound healing

- EGF
- Inhibitor
- Fibroblast proliferation, migration, ECM synthesis
- Keratinocyte proliferation, differentiation
- Angiogenesis

Diagram shows a process involving various growth factors and their interactions in the wound healing process. Key factors include TGF-β, VEGF, FGF, and PDGF, among others. The diagram illustrates the roles of these factors in different stages of wound healing, such as inflammation, proliferation, and remodeling.
## Growth factors in wound healing

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<th>Process</th>
<th>Growth Factors</th>
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<td>Epithelial proliferation</td>
<td>EGF, TGF-α, KGF, HGF</td>
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<tr>
<td>Monocyte chemotaxis</td>
<td>PDGF, FGF, TGF-β</td>
</tr>
<tr>
<td>Fibroblast migration</td>
<td>PDGF, FGF, TGF-β</td>
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<tr>
<td>Fibroblast proliferation</td>
<td>PDGF, EGF, FGF, TNF</td>
</tr>
<tr>
<td>Angiogenesis</td>
<td>VEGF, Ang, FGF-2</td>
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<tr>
<td>Collagen synthesis</td>
<td>TGF-β, PDGF</td>
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<td>(TGF-β3 inhibits)</td>
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**EGFR**

Skin

**BM stroma (myelofibrosis)**

*Chemokines, cytokines are also involved in the inflammatory phase of regeneration, which are not discussed here, only their receptor types were shown.*
Extracellular matrix (ECM) and cell-matrix interactions

ECM is a dynamic continuously remodelled complex of macromolecules

ECM not an inert material just for filling up gaps !!!

Functions:
Sequesters water and provides turgor (resistance) for tissues
minerals (Ca$_{10}$(PO$_4$)$_6$(OH)$_2$ hidroxil-apatit, for bone rigidity

Supports (and anchores) parenchymal cells and their migration
Basement membrane (BM) in kidney is involved in glomerular filtration

Binds and offers regulatory molecules (growth factors)
for: cell proliferation, migration, differentiation

Initiates signal transduction through integrins
They can be transmembrane molecules too (syndecan, collagen XVII)

Active contributors of wound healing

Types: Interstitial matrix
Basement membrane
Extracellular matrix (ECM)

1. Fibrillar proteins: collagens, elastin (elastase – Ilona Banga)
2. Water-hydrated gels: Proteoglycans, hyaluronan
3. Multiadhesive proteins: fibronectin, laminin

Signals from the matrix through integrins: laminin, fibronectin, collagens
Heparan sulfate proteoglycans: RTK receptor coreceptor
ECM signaling through integrins

Laminin fibers

EXTRACELLULAR MATRIX

Laminin fibers

Collagen

Fibronectin

Growth factors

Growth factor receptor

β

Integrin

α

β

Integrin

FOC

Growth factors

Growth factor receptor

Focal adhesion complexes

Actin cytoskeleton

CYTOSKELETAL-MEDIATED SIGNALS

CYTOPLASMIC SIGNAL TRANSDUCTION PATHWAYS

Cytoplasmic signal transduction pathways

Nucleus

PROLIFERATION, DIFFERENTIATION, PROTEIN SYNTHESIS, ATTACHMENT, MIGRATION, SHAPE CHANGE

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ECM binds and offers growth factors for cell activation
Timing of main events in wound healing

Granulation tissue
- Early fibroblasts & ECM
- Network of newly formed vessels
- Inflammatory cells (macrophages)

Trichrome staining
Collagen - blue

Cells Involved in Wound Healing
Re-epithelialization

Epidermal keratinocytes produced from interfollicular (basal) and follicular (bulge region) stem cells migrate from both edges of wound

Keratinocytes migrate over the transitional wound matrix and fibrin cloth (including early matrix components e.g. fibronectin)

Covering the wound surface below scab, keratinocytes differentiate into stratified squamous „neoepidermis” which usually show hyperplasia

Re-epithelialization is phylogenetically a robust feature under most conditions

Control – „Normal” wounding  C-Met (HGFR) mutant

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<td>HE</td>
<td>G</td>
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<tr>
<td>F</td>
<td>Es</td>
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Delayed re-epitelialization

-epithelial hyperplasia

Formation of new vessels
- Vasodilataion, endothelial cells budding (sprouting), proliferation
- Progresses toward wound space (hypoxia gradient & growth factors), release of pericytes
- Immature vessels differentiate into capillaries, arterioles, and venules (regain pericyte support)

Upon angiogenic stimuli e.g. by macrophages (VEGF, FGF) and keratinocytes (VEGF) PDGF, Angiogenin (maturation)
Fibroplasia – Matrix production

**Fibroblasts** (PDGF, FGF-2, TGF-β1 –β2, TGF–β3)
- Migrate into wound site and replicate (*stabile cells*)
- Dominant cell type at wound edge
- Synthesize and deposit collagen and proteoglycans

- **Matrix deposition** depends on oxygen and substrate availability and regulated by growth factors
- Cross linking of collagen is important for tensile strength, which requires vitamin C
- Vitamin C-deficiency: skeletal deformities, hemorrhages and retarded wound healing

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**Loose connective tissue**

- Mesenchymal cell
- Elastic fibers: fibrillin scaffold + elastin
  - Marfan syndrome: fibrillin mutations
    - long thin body, long extremities
    - highly flexible joints (shoulder, elbow, ankle)
    - extra long wingspan: (>1.05 of height)
  - Aorta dissections: detachment of the aorta layers

![Image of Michael Phelps](image)
Epithelial migration - Matrix remodelling

MMPs (Zn\(^{2+}\)):
Collagenases, Gelatinases, Strome-lyzines

Cleavage of precursors results in new biological activity
(controlled proteolyzis – bioactive molecular fragments)

**Martricryptic sites**

Collagen XVIII - endostatin
Perlecan - endorepellin

angiogenezis inhibitors
Types of healing

Identical steps, but in the secondary:
- defect is larger
- wound edge is wider
- extensive inflammation
- healing is slower
- scar is larger
- substantial contraction
Healing of special tissues

Dependent from:  
- type of tissue
- regeneration potential
- level of ECM damage

Liver:  
- regeneration (focal, zonal necrosis)
- damage of ECM network (large abscess, cirrhosis) healing by fibrosis

Hepatocytes + oval cells

CK7+ Hering ducts  
(oval cells are stem cells)
The pathway of healing is identical with that in the skin but faster

Skin transplanted into the oral cavity - wound - healed with scar

Differences:
(inherent)
- **less inflammatory cells** (granulocytes, macrophages)
- **saliva**: antibacterial protection, accelerated clotting (exosomes) & proliferation (EGF), leukocyte protease inhibitor, histatins
- **less protocollagen I and fibronectin**, more tenascin
- residens fibroblasts express **less TGFβ1** and decorin (fibrosis)
- **less** fibrillogenesis and **myofibroblasts** (contraction)
Regeneration of bone fracture

**Trauma**
- Bleeding, coagulation (fibrin)
- Inflammation, Fibroblasts
- PDGF

**Bone and cartilage progenitor cells**
- (chondrocytes & osteoblasts)
- From periosteum & bone marrow

**Synthesis of new matrix**
- Soft callus (cartilage)

**Endochondrial ossification**
- (similar to that of epiphyseal growth plate - calcification)

**Woven bone – lamellar bone**
- Trabecular bone: Remodelling
Factors influencing wound healing

Local: type of tissue, blood supply (nutrients, oxygenization), mechanical stress, localization & type of injury (cut, large destruction, acid, alkaline, burn) irradiation: UV, radioactive (ionizing) etc…

Systemic: cardiovascular status, infections, nutrition (malnutrition), diabetes, lack of vitamins (e.g. vitamin C), corticosteroids

Complications
Large tissue loss, deep wound – substantial scarring and distortion
Pressure ulcer (decubitus): insufficient perfusion and oxygenization
mechanical pressure > resistance of arterial wall
**Insufficient closure, dehiscence:** mechanical stress/trauma, increased abdominal pressure due to coughing or vomiting; Risk: diabetes, obesity, poor suture
Old age, poor perfusion: poor oxygenization, mechanical stress, diabetes
Chronic wound (diabetic ulcer): arteriopathia, reduced perfusion, oxygen and nutrients, insufficient immune response against infection, neuropathy
Enzymes of the larvae digest dead tissue at wound edges which is then eaten up by the larvae.

Support the elimination even of antibiotic resistant bacteria: Staphylococcus aureus (MRSA) or Streptococcus B.
**Keloid**: elevated collagen synthesis, lost control during healing, inherited, more frequent in the African-American population.
Contracture of palmar fascias: (Dupuytren contrature) restricted mobility/extendibility of fingers due to elevated fibrosis and shortening of fascia around finger tendons (post-trauma)

Oesophagus stricture

10% NaOH kontroll

Osman et al. 2008
(alkali drinking - suicide)
Colliquation necrosis
Regeneration-Healing

Coordinated interactions between cells, ECM, growth factors, enzymes (proteases, protein kinases).
Thank you!

Lung fibrosis – Trichrome staining (blue = collagen)