Gingival Wound Healing

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GOAL OF WOUND HEALING

• Goal of skin and mucosal wound healing is to restore the barrier function of the tissue as fast as possible to prevent microbial access into the tissue.

• In ideal situation, wound healing also leads to complete structural regeneration of disrupted tissue and restoration of function.

• Ideal wound healing also leads to aesthetically satisfactory tissue architecture.

• However, tissue disruption in higher vertebrates, unlike lower vertebrates, results most often not in tissue regeneration, but in a repair process leading to a fibrotic scar.
Wound healing, whether initiated by trauma (such as surgery), microbes or foreign materials, proceeds via overlapping pattern of events including:

- Haemostasis and inflammation (coagulation)
- Re-epithelialization
- Formation of granulation tissue
- Tissue remodeling

Wound healing process is a dynamic, highly regulated process, where soluble mediators (cytokines, chemokines, growth factors, bioactive peptides) and extracellular matrix molecules orchestrate the function of inflammatory cells, epithelial cells, endothelial cells and fibroblasts.
PHASES OF WOUND HEALING AND CELL TYPES INVOLVED IN EACH PHASE

1. INFLAMMATION: Haemostasis (within minutes) and early and late inflammation (day 0-7)
   Platelets, PMNs, Macrophages, Lymphocytes, Mast cells

2. RE-EPITHELIALIZATION: a) Migration of epithelial cells to cover the wound space (day 1-7); b) epithelial differentiation and reformation of basement membrane (up to 21 days)
   Epithelial cells (keratinocytes)

3. GRANULATION TISSUE FORMATION (day 3-14)
   Macrophages, Granulation tissue fibroblasts, Myofibroblasts, Endothelial cells

4. TISSUE REMODELING: Wound contraction, matrix reorganization and remodeling and normalization of cellularity (from day 5 up to 1-2 years)
   Fibroblasts, Endothelial cells
SEQUENCE OF WOUND HEALING

Haemostasis & Inflammation

Granulation tissue formation

Tissue remodeling

LATE PHASE

Wound contraction

Collagen accumulation

EARLY PHASE

Re-epithelialization

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<th>Time (days)</th>
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% of Maximum Response
Wound healing studies by the UBC Periodontal Biology Laboratory

- Experimental wounds in human gingiva
- Extracellular matrix molecules produced by migrating gingival keratinocytes
- Expression and function of keratinocyte matrix receptors, integrins
- Regulation of cell adhesion and migration
- Cell signaling cascades that regulate cell migration
- Gene chip analysis of gene expression during wound healing
Healing of experimental gingival wounds after 3, 4, and 7 days and after 3 months
3-day
Re-epithelialization

7-day
Granulation tissue formation

14-day
Wound contraction

28-day
Remodeling
Epithelial cells migrate through the clot in gingival wounds

Migrating epithelial cells are highly phagocytic and able to digest fibrin
DURING RE-EPITHELIALIZATION, CELL FUNCTIONS ARE REGULATED BY:

1) SOLUBLE MEDIATORS RELEASED FROM CELLS, BLOOD AND EXTRACELLULAR MATRIX (ECM):

- CYTOKINES
- CHEMOKINES (chemoattractant cytokines)
- GROWTH FACTORS
- BIOACTIVE PROTEINS OR PEPTIDES RELEASED FROM CELL MEMBRANES OR ECM BY PROTEOLYSIS

THESE MOLECULES EXERT THEIR FUNCTION THROUGH SPECIFIC CELL-MEMBRANE RECEPTORS IN THEIR TARGET CELLS.

2) ECM MOLECULES

CELLS CAN RECOGNIZE CHANGES IN THE COMPOSITION OR ORGANIZATION OF ECM MOLECULES BY INTEGRAL CELL MEMBRANE MOLECULES, INCLUDING INTEGRINS AND CELL SURFACE PROTEOGLYCANS.
Migrating keratinocytes express **EDA** Fibronectin (A, C) while **EDB** Fibronectin (B, D) is specifically expressed in the granulation tissue.
TGFβ induces keratinocyte migration and expression of EDA Fibronectin (C, D, E, F). Control cells migrate slowly (A, B)
Expression of Tenascin is induced in 7-day-old wound granulation tissue
Expression of TN-L (Large variant) in wounds
Migrating epithelial cells express Laminin-5

-Laminin-5 (LM-5) is a component of the anchoring filaments of the hemidesmosome.

-LM-5 can be proteolytically processed either to support cell migration or stop the cells from migration by formation of stable anchoring structures, hemidesmosomes.
TGFβ1 stimulates expression of both EDA Fibronectin and LM-5
Epithelial cell integrins

- $\alpha_2\beta_1$ collagen receptor
- $\alpha_3\beta_1$ laminin-5 receptor
- $\alpha_6\beta_4$ laminin-5 receptor
- $\alpha_9\beta_1$ tenascin receptor
- $\alpha_5\beta_1$ fibronectin receptor
- $\alpha_\nu\beta_1$ fibronectin receptor
- $\alpha_\nu\beta_6$ tenascin receptor
Expression of integrins in wounds

0 days

α2β1
α3β1
α6β4
α9β1

αvβ1
αvβ6
α5β1
Expression of beta 1 integrins in 3-day-old gingival wound

- High expression of integrins at the leading edge
- Migrating epithelial cells
- Fibrin clot
Expression of laminin-5 receptor, alphav beta4 integrin, in gingival wounds
Extracellular matrix molecules and integrins expressed by migrating keratinocytes in 3-day-old wounds

\[ \alpha_2\beta_1 \]
\[ \alpha_3\beta_1 \]
\[ \alpha_5\beta_1 \]
\[ \alpha_9\beta_1 \]
\[ \alpha_{\nu}\beta_1 \]
\[ \alpha_6\beta_4 \]

LM5 +++
TN +++ (large and small variant)
FN ED-A +++
TGFβ1 promotes concentration dependent cell adhesion and migration of keratinocytes on fibronectin
Matrix metalloproteinases (MMPs) play an important role during wound healing.
WOUND RE-EPITHELIALIZATION: SUMMARY

Release of cytokines, growth factors and bioactive proteins and peptides from blood clot, damaged cells and inflammatory cells

**Activation of epithelial cells at the wound edge:**

First epithelial cells migrate into the fibrin clot about 24 h after wounding:
- Basal keratinocytes dissolve hemidesmosomes to release themselves from BM
- Keratinocytes secrete proteases that help to detach the cells from BM and to modify ECM to facilitate migration
- Keratinocytes are induced to express new integrins that mediate cell adhesion to the matrix of the blood clot
- Keratinocytes are induced to express endogenous growth factors that enhance cell migration
- During migration, keratinocytes produce their own ECM molecules that they use as a migration substrate (laminin-5, fibronectin, tenascin)
- Keratinocytes behind the migrating cells undergo cell proliferation.
Schematic view of epithelial cell migration

A. wounding → stopping → braking → migrating

B. TGFβ

Legend:
- LAMINA DENSIA
- LM-1, TYPE IV COLLAGEN, HSPG
- α6β4
- PROCESSED LM-5
- α3β1
- UNPROCESSED LM-5
- uPAR/PAI-1
- EDA-FN
- α5β1 (αvβ1, αvβ6)
- α2β1
- EDA/EDB-FN
- αvβ6
- LAP-TGFβ
When epithelial cell fronts migrating from opposite sites of the wound meet each other at the mid-wound, cell migration stops around day 7.

Keratinocytes differentiate and form stratified epithelium.

The reformation of basement membrane is also initiated by secretion of type IV and VII collagen and laminin-1 and -5 and heparan sulphate proteoglycans by keratinocytes. Fibroblasts also participate to produce some of the BM proteins. The adhesion of epithelial cells to the BM is reestablished by formation of new hemidesmosomes. Basement membrane is regenerated around day 21.
Molecular wound healing is still incomplete at 4 weeks

3-days
BM lacking underneath migrating cells, no GT

7-days
BM, GT deposition

14-days
Maturation of BM, GT

28-days
BM normalized, wound specific integrins and GT molecules still present
Connective tissue bridge is formed under recently fused epithelium

7-day-old wound:

- wound clearly visible
- fibrin still present in the granulation tissue
- collagen is first deposited by granulation tissue fibroblasts under the fused epithelium and in the deep connective tissue
Epithelial regulation of connective tissue formation: $\alpha_\nu\beta_6$ Integrin

- exclusively epithelial cell integrin
- Fibronectin/tenascin receptor
- induced during late wound healing and invasive front of SCC
- modulates epithelial-driven inflammation
- binds to latent- TGF$\beta_1$ (higher affinity than FN) and activates it
- Anti- $\nu$ antibodies prevent exp. kidney fibrosis
αvβ6 integrin and GT formation

Activation of TGFβ1

Myofibroblast differentiation and matrix production
Expression of alphavbeta6 integrin and collagen in human chronic wounds

Diabetic ulcer

Decubitus ulcer

Chronic leg ulcer

Alphavbeta6 integrin

Procollagen I

HE
Expression of alphavbeta6 integrin in beta6 overexpressing mice
Expression of alpha_vbeta_6 integrin in wounds of normal (A, C, E) and overexpressing (B, D, F) mice.

3-day wounds

7-day wounds

28-day wounds
Overexpression of alphav beta6 integrin leads to chronic scarring in mice

Transgenic mice were created that overexpress β6 integrin under Keratin-14 promoter (specific expression in basal keratinocytes). A-C, different types of chronic scars
Scar tissue in alphavbeta6 overexpressing mice is full of macrophages.

Beta6 integrin in situ
Beta6 immunostaining
SMA

PTA
HE
TGFbeta
Macrophages
MAJOR CYTOKINES AND GROWTH FACTORS PRODUCED BY MACROPHAGES

- TNF-\(\alpha\)
- IL-1
- IL-6
- IL-8
- IL-10
- IL-12
- MIP-1
- MIP-2
- IFN-\(\gamma\)
- PDGF-A/B
- TGF-\(\alpha\)
- TGF-\(\beta\)
- aFGF / bFGF
- Heparin-binding epidermal growth factor
- IGF-1

MACROPHAGE
Cellular signaling during epithelial cell migration

• extracellular matrix integrins alpha5beta1, alphavbeta1 and alphavbeta6 bind to fibronectin in the wound provisional matrix that leads formation of long cell extension called lamellipodia and subsequent cell migration

• cellular signaling pathways that regulate lamellipodia formation have remained virtually unknown
Migrating gingival keratinocytes extend long cellular projections (lamellipodia) into the wound provisional matrix
Cultured keratinocytes express long lamellipodia when exposed to Epidermal Growth Factor but this process is slow (24-48 h)

A, Wound keratinocytes

B,C: Cultures keratinocytes exposed to EGF
A broad spectrum serine/threonine kinase inhibitor, staurosporine, can rapidly (1 h) induce extended lamellipodia (E-Lams) formation in cultured keratinocytes.
Extended Lamellipodia (E-Lams) formation by Staurosporine is concentration dependent.
Lamellipodia formation requires tyrosine phosphorylation

Control Staurosporine

A  B

Control

C  D
Sodium orthovanadate

E  F
Herbimycin A

G  H
Genistein
Lamellipodia formation requires cytoskeletal organization by **actin** and **tubulin**.
Lamellipodia formation requires normally regulated small GTPase, Rac
Lamellipodia are dependent on PI3K, PLCγ, and intracellular Ca

Phosphoinositol-3-kinase inhibitor LY-294002 (µM)

Chelation of intracellular Ca by BAPTA-AM

Inhibition of Phospholipase Cγ by U-71322 (µM)
Staurosporine induces dephosphorylation of Serine21 residue in **Glycogen Synthase Kinase-3 (GSK-3)** and phosphorylation of Tyrosine residues that are consistent with **GSK-3 activation**
LiCl2 and a specific inhibitor of GSK-3 (SB-415286) block Staurosporine-induced lamellipodia formation

A

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<th>Control</th>
<th>Staurosporine</th>
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Number of cells spread, %

B

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<th>SB-415286 (µM)</th>
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Number of cells spread, %

a, cells exposed to Staurosporine; b, cells exposed to Staurosporine and the GSK-3 inhibitor (SB-415286)
Cellular signaling pathway regulating lamellipodia formation in keratinocytes (Staurosporine induced)

LY-294002 → PI3K

↓

Rac

↓

*PLC γ

U-71332

↓

Increase in intracellular Calcium

↓

*GSK-3

LiCl₂

SB-415286

↓

Extended lamellipodia formation

PI3K = phosphoinositol-3-kinase
Rac = small GTPase
PLC = Phospholipase
GSK = glycogen synthase kinase