Synergy in microenvironmental impact between matrix proteins & growth factors on human mesenchymal cells

Henry C. Hsia, M.D.
Vivek Desai, B.S.
Jean Schwarzbauer, Ph.D.

10th New Jersey Symposium on Biomaterials Science
New Brunswick, NJ
October 28, 2010
The extracellular matrix regulates many biological processes

Stem cells & Regenerative Medicine

Cancer

Development

BodyFig1C

Adult hermaphrodite

Tail (tapered)

Oocyte in gonad

Gonad

Vulva

Embryos

0.1mm

Courtesy of Jean Schwarzbauer, Princeton University
How does the extracellular matrix control wound healing?

From Monty Python and the Holy Grail (1975)
Wound healing is unpredictable

Normal

Abnormal

hypertrophic scar

keloid
Normal wound healing

0 (weeks) 1 2 3 >12

Injury Scar

Wound Contraction

Remodeling

Matrix deposition (Fibronectin)

Collagen scar

TGF-β

Myofibroblasts (αSMA)

Fibrin matrix
TGF-β initiates myofibroblast differentiation

Adapted from Hinz, B. et. al. Am J Path 2007;170:1807-16
Abnormal wound healing

Injury

0 (weeks) 1 2 3 >12

Scar

Remodeling

Proliferation

TGF-β

Wound Contraction

Myofibroblasts (αSMA)

Tenascin-C

Matrix deposition (Fibronectin)

Fibrin matrix

Collagen scar

Inflammation

Scar

Matrix deposition (Fibronectin)

Collagen scar

0 (weeks) 1 2 3 >12

Injury

Scar

Abnormal wound healing

Abnormal wound healing

Abnormal wound healing

Abnormal wound healing

Abnormal wound healing

Abnormal wound healing

Abnormal wound healing

Abnormal wound healing
Fibronectin (FN) mediates cell adhesion by interacting with α5β1 integrin & syndecan-4.
Tenascin-C: a highly conserved ECM protein upregulated during tissue remodeling
Tenascin-C is highly expressed at wound edges

Stained tissue section of a cutaneous wound

Blood clot with cross-linked fibrin & fibronectin (provisional matrix)

Modified from Martin (1997) Science 276, 75-81
3D provisional matrix allows study of wound cell behavior in vitro

- Fibrinogen
- FN (fibronectin)
- Calcium (Ca++)
- Factor XIII
- +/- Tenascin-C
- Thrombin

Cross-linked fibrin-fibronectin clot
Tenascin-C competes with syndecan-4
Syndecan-4 overexpression reduces cell response to tenascin-C

(rat fibroblasts; Scale bar, 60 µm, inset, 10 µm)
Cell contractility can be assessed using a 3D matrix contraction assay
Cell contractility can be assessed using a 3D matrix contraction assay.
Syndecan-4 overexpression reduces inhibition of cell-mediated matrix contraction by tenascin-C
Through syndecan-4, tenascin-C alters synergistic cell signaling by FN

Through syndecan-4, tenascin-C alters synergistic cell signaling by FN
Tenascin-C & myofibroblast differentiation

0 (weeks)  1   2  3  >12

Injury

Inflammation

Proliferation

Remodeling

Scar

Matrix deposition (Fibronectin)

Wound Contraction

Myofibroblasts (αSMA)

Tenascin-C

Provisional matrix

Collagen scar

TGF-β

ECM (fibronectin)

focal adhesions

actin stress fiber

α-smooth muscle actin
Alpha-smooth muscle actin is a marker of TGFβ-mediated myofibroblast differentiation.

Total actin

α-SMA

(human fibroblasts)
Tenascin-C treatment enhances TGFβ-mediated expression of α-SMA

(human adipose-derived stromal cells: green=total actin; red=α-SMA)
Treatment with TGF-β & tenascin-C leads to synergistic impact on αSMA expression levels

(cells stained after 4 days treatment)
Treatment with TGF-β & tenascin-C leads to synergistic impact on αSMA expression levels

(cell lysates after 2 days treatment)
More robust focal adhesion formation with TGF-β1 and tenascin-C

Untreated

Red = α-SMA
Green = vinculin

bFGF

TGF-β1+tenascin-C

Total vinculin (140 kD)

(Images taken at 60x)
TGF-β and tenascin-C have a synergistic impact on cell-mediated contraction of a 3D matrix

(ADSCs after 4 days of treatment as indicated)
Dual roles for tenascin-C in the wound microenvironment

- Fibrin matrix
- Myofibroblasts (αSMA)
- Wound Contraction
- TGF-β
- Matrix deposition (Fibronectin)
- Tenascin-C
- Collagen scar

Injury: 0 (weeks) → 1 → 2 → 3 → >12

Scar: Remodeling

Proliferation
Dual roles for tenascin-C in the wound microenvironment

- FAK
- Rho

↓

- Focal adhesions
- Actin stress fibers
- Matrix contraction

↓

? asked

↑

- Focal adhesions
- \(\alpha\)-SMA stress fibers
- Matrix contraction
Acknowledgements

- Department of Molecular Biology, Princeton University
- Department of Surgery, UMDNJ-Robert Wood Johnson Medical School
- The New Jersey Center for Biomaterials
- National Institutes of Health:
  - K08-GM072546 (HCH)
  - R01-CA044627(JES)
Thank you!