Effects of exudate physical, chemical and thermal conditions on collective migration of tissue-repairing cells: Mechanobiological studies

Amit Gefen, Ph.D.

Professor of Biomedical Engineering
The Herbert J. Berman Chair in Vascular Bioengineering

Department of Biomedical Engineering
Faculty of Engineering
Tel Aviv University
Email: gefen@tauex.tau.ac.il

Past President
European Pressure Ulcer Advisory Panel (EPUAP)

LINK®
Wound Healing
Congress 2019

TEL AVIV UNIVERSITY אוניברסיטת תל אביב

LINK®
Learn. Inform. Network. Knowledge.

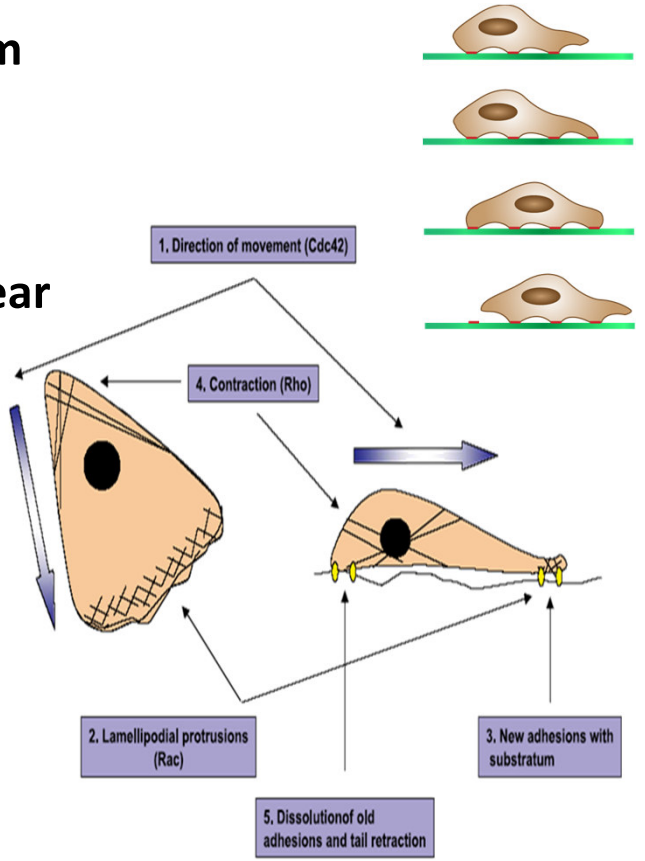
September 11th 2019, Budapest

Mechanisms of cell migration as related to wound healing

***(i)* Extension of a leading edge protrusion - lamellipodium**

***(ii)* Establishment of new adhesion sites at the front**

***(iii)* Contraction & detachment of adhesions at the cell rear**



Raftopoulou and Hall, *Developmental Biology*, 2004

Proteins which control the cytoskeleton:

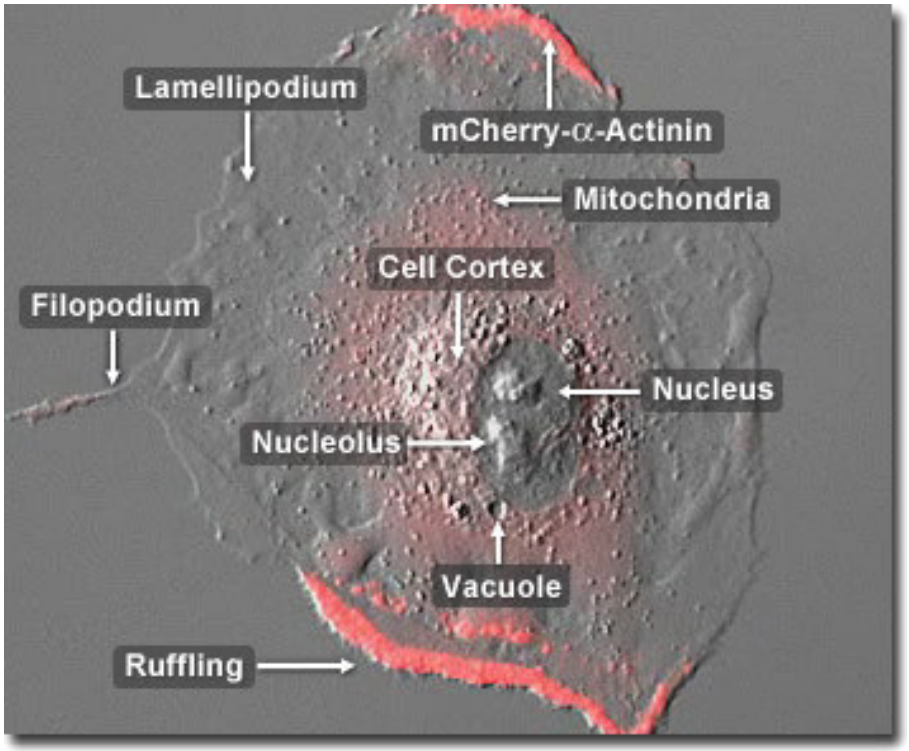
- Rac: Promotes formation of actin polymers at the lamellipodium
- Rho: Controls formation of actin-based cytoskeletal structures
- Cdc42: Controls cell polarity & migration direction in wounds

* Migration control balances activation of

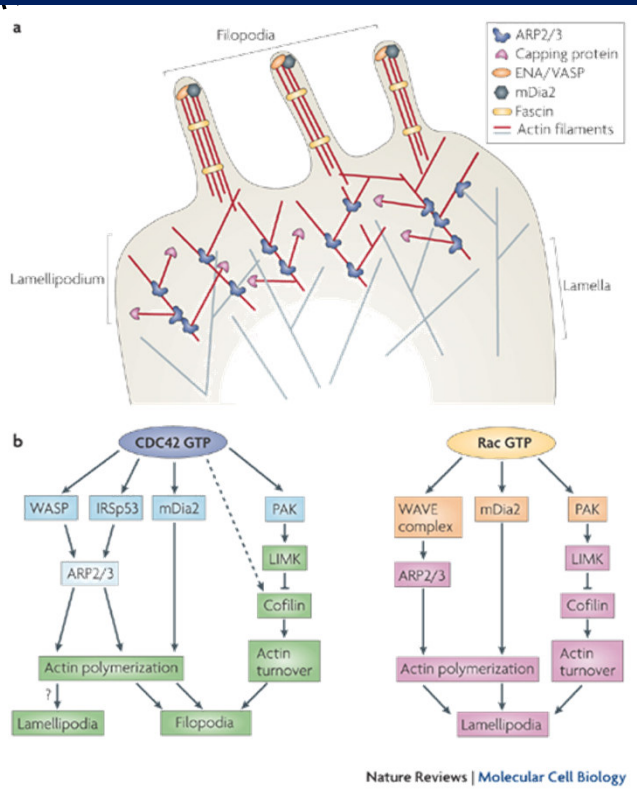
Rac ⟺ **Rho**

stimulates cell spreading/migration *cell contractility and adhesion*

Cell movement versus probing functions



http://www.olympusmicro.com

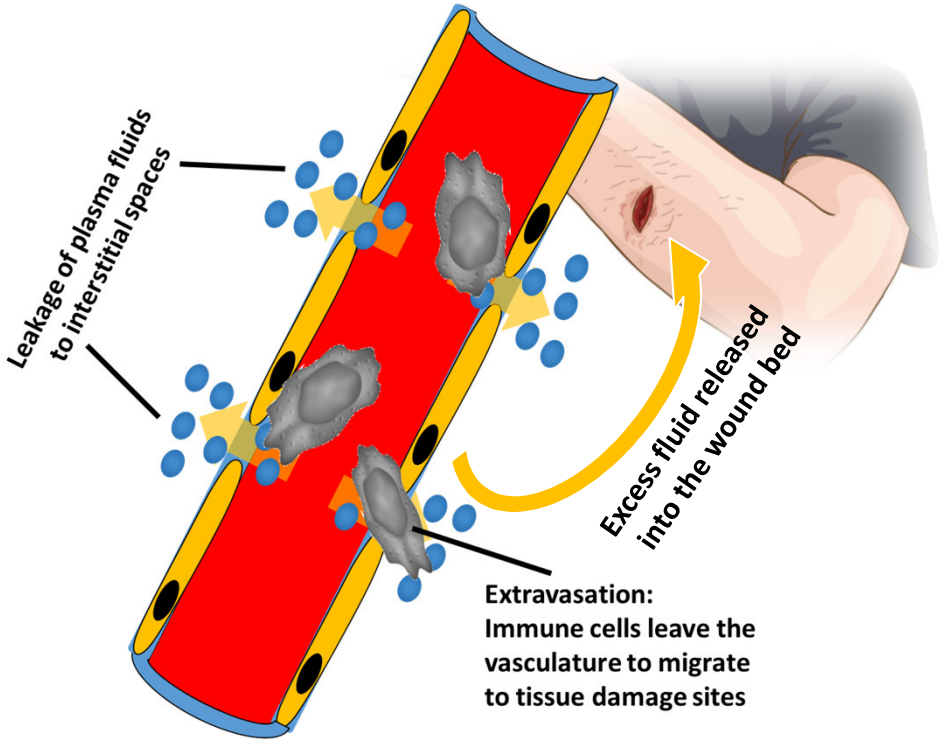


Heasman and Ridley *Nature Reviews Molecular Cell B*iology , 2008

- Lamellipodia are broad sheet-like protrusions controlled by Rac that contain a branched network of short actin filaments
- Filopodia are long, thin protrusions regulated by Rho/Cdc42 that emerge from the plasma membrane and allow cells to probe

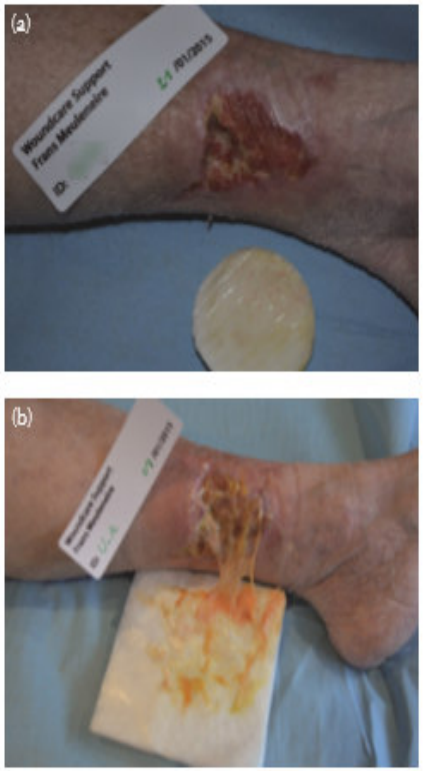
The exudate is the environment in which cells migrate in the wound bed

Source of exudate fluids:
Leaky vessels due to inflammation



Gefen *Med Eng Phys*, 2019 (under review)

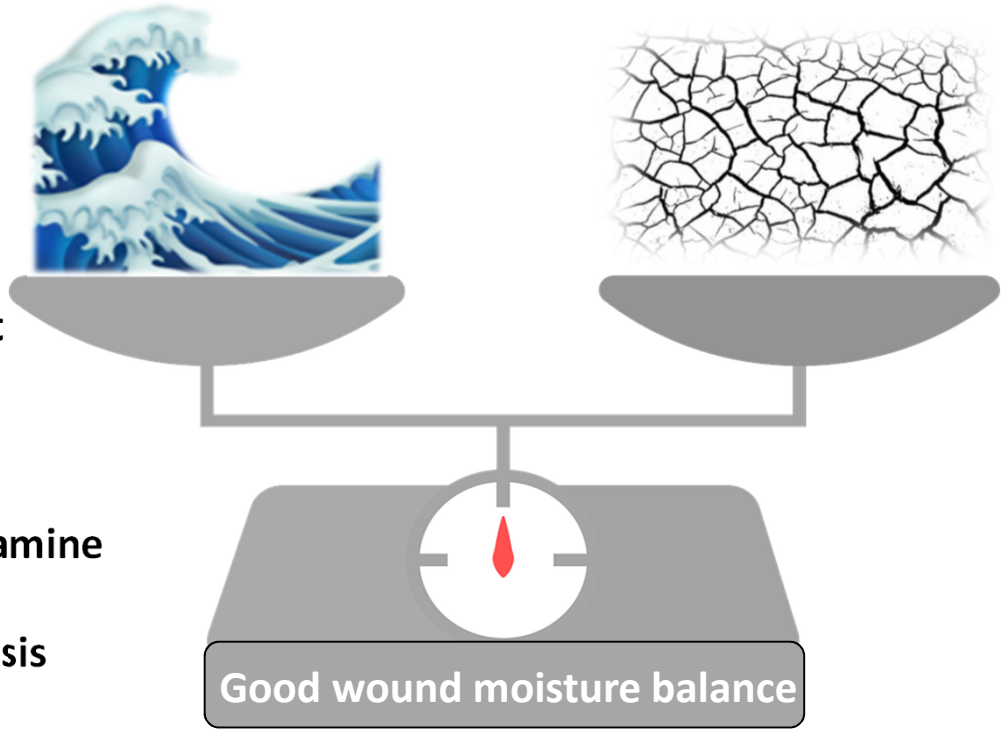
Compositions & properties of wound exudates vary considerably



Vowden et al. *Wounds UK* 2015

The roles of exudate in wound healing

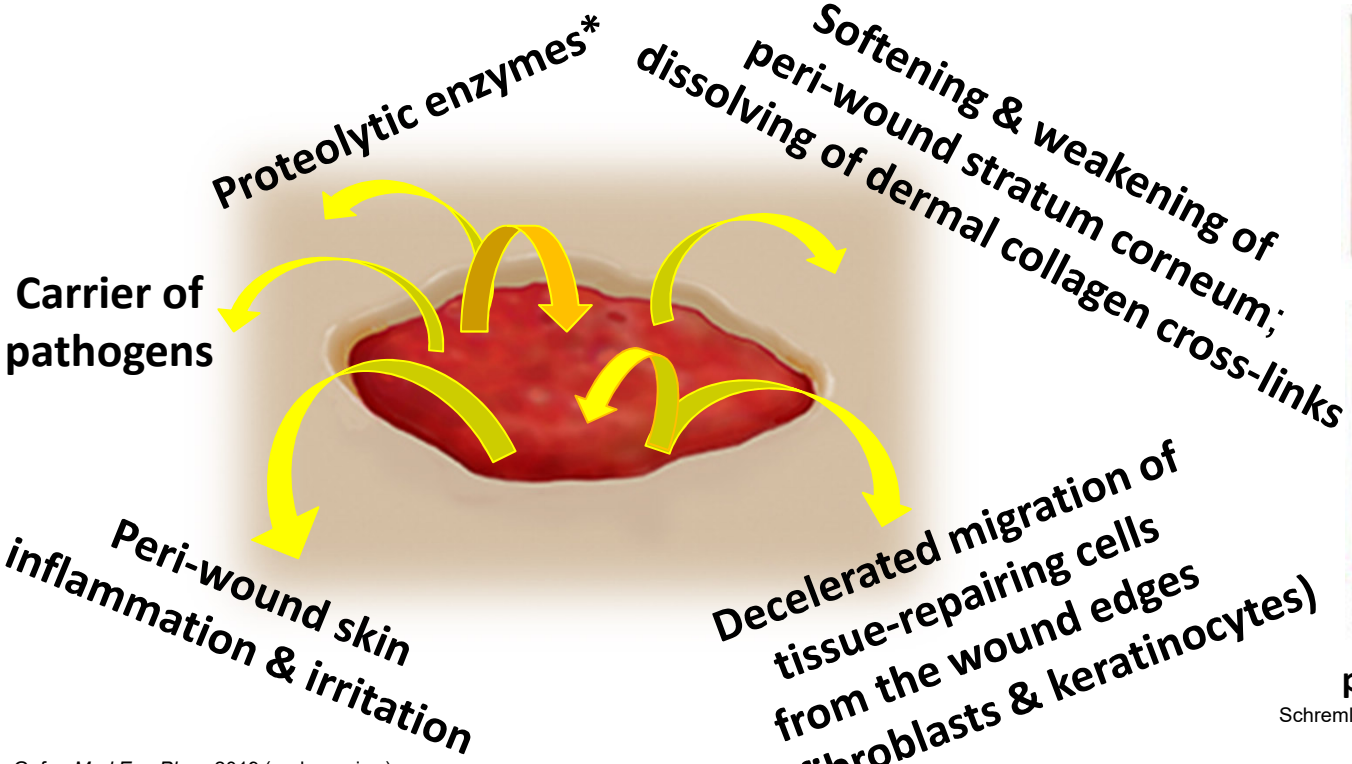
Moisture (presence of exudate) in the wound bed is critical for:



- **Preventing the wound bed from drying out**
- **Nutrient diffusion to cells**
- **Inflammatory mediator diffusion, e.g. histamine**
- **Growth factor diffusion, e.g. for angiogenesis**
- **Allowing immune cell migration → reduce bacterial burden**
- **Ultimately, allowing migration of tissue-repairing cells, e.g. fibroblasts**

Gefen *Med Eng Phys*, 2019 (under review)

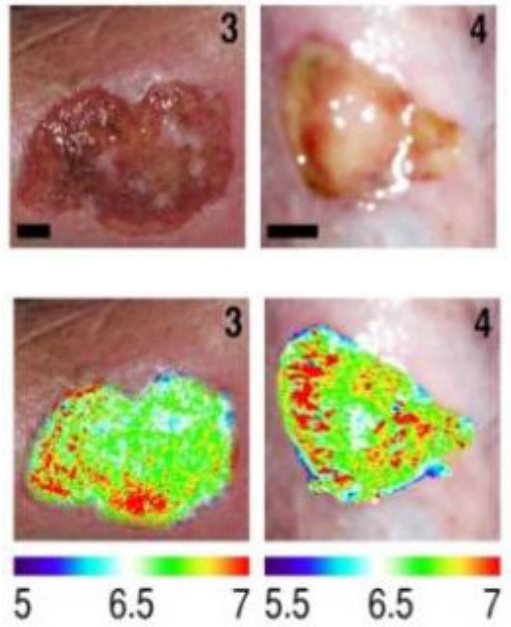
Hostile-to-cells wound exudates can hinder cell migration & repair



Gefen *Med Eng Phys*, 2019 (under review)

* particularly in chronic ulcer exudates

Palolahti et al. *Exp Dermatol.* 1993



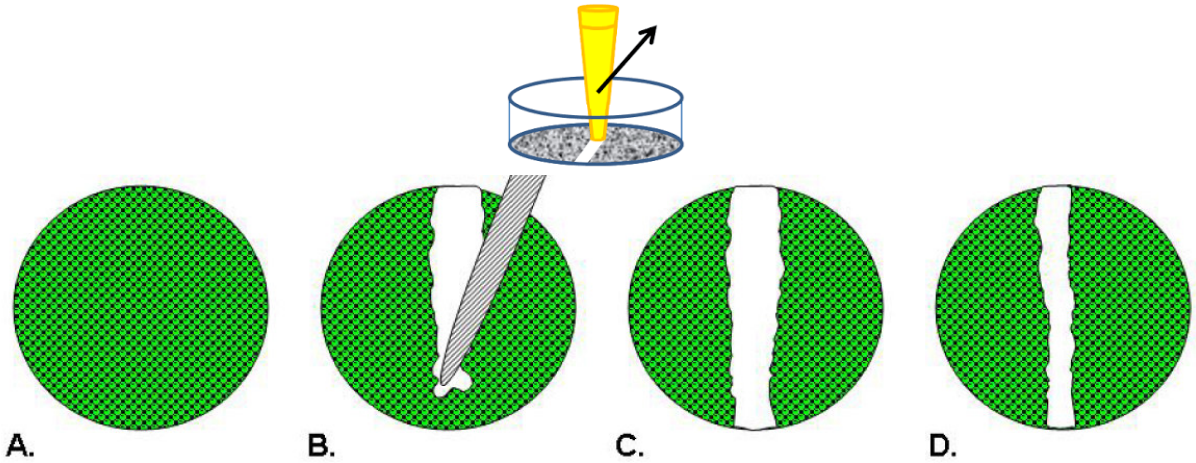
pH of exudates tends to be acidic

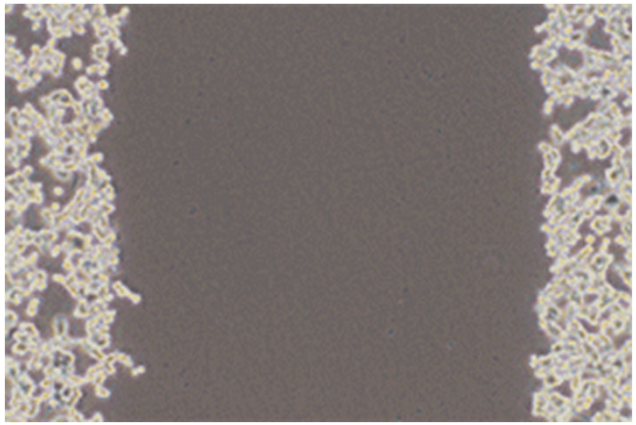
Schreml, *European Journal of Molecular & Clinical Medicine* 2015

Gefen *Journal of Tissue Viability* 2011
Topman, Gefen et al. *Ann Biomed Eng.* 2012
Woo et al. *Advances in Skin & Wound Care* 2017
Kottner, Gefen et al. *Clin Biomech.* 2018
Schwartz, Gefen et al. *Int Wound Journal* 2018

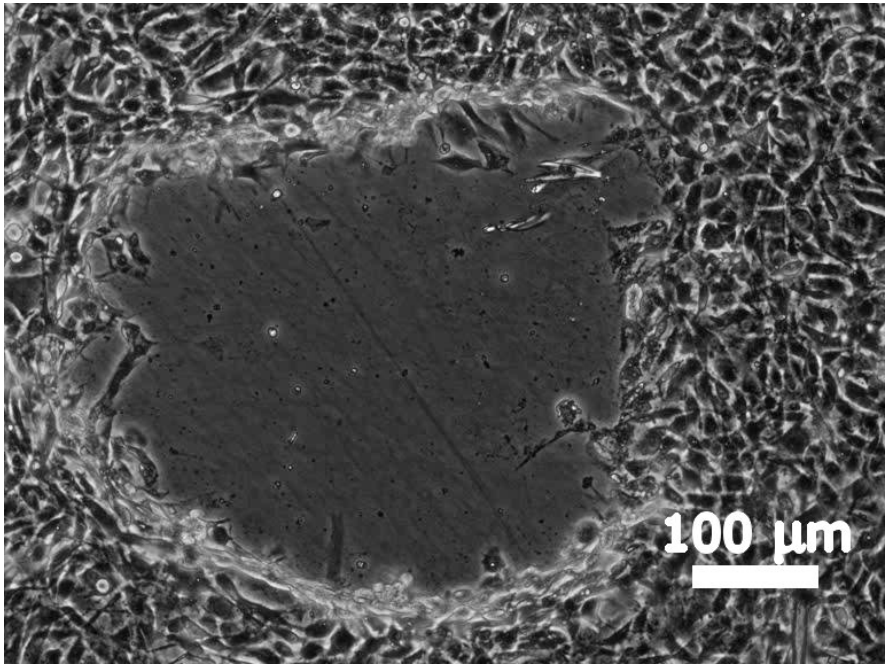
Studies of the impact of the chemical and physical exudate environment on collective cell migration

Measuring collective cell migration: Traditional scratch assays





Measuring collective cell migration: Assay automation (I)

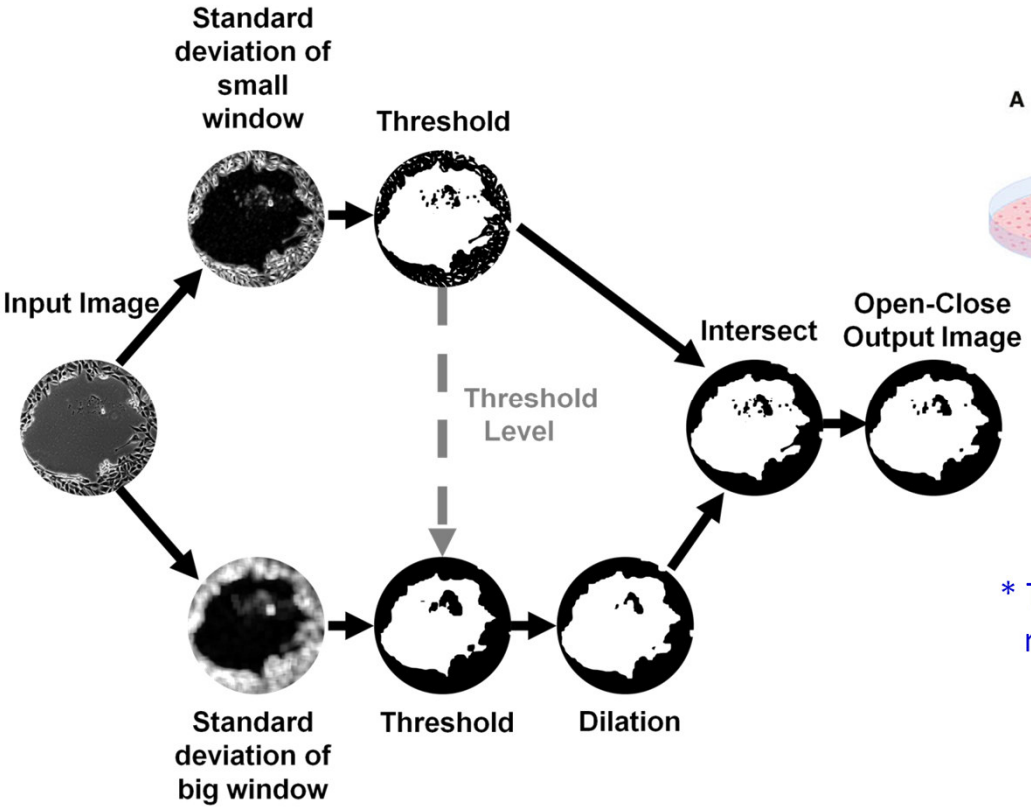


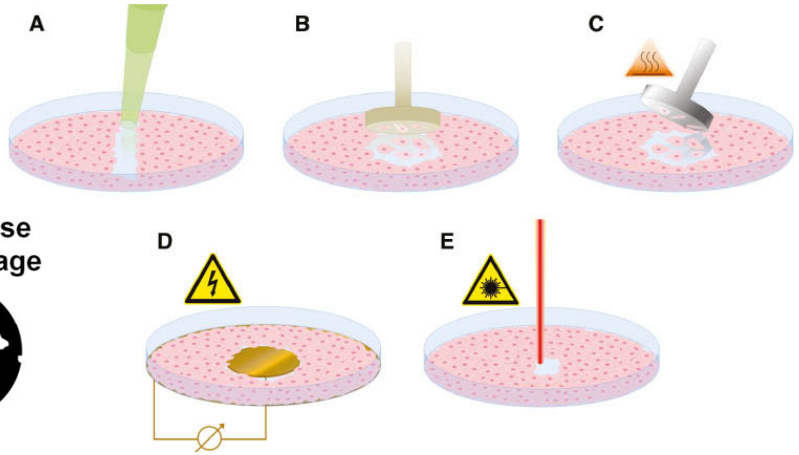
- The “wound” area has a more homogenous localized image texture
- Standard deviation of pixel intensities in a window is a texture homogeneity measure

Topman et al. *Med Eng Phys*, 2012

Measuring collective cell migration: Assay automation (II)

Schematics of the wound area detection algorithm:

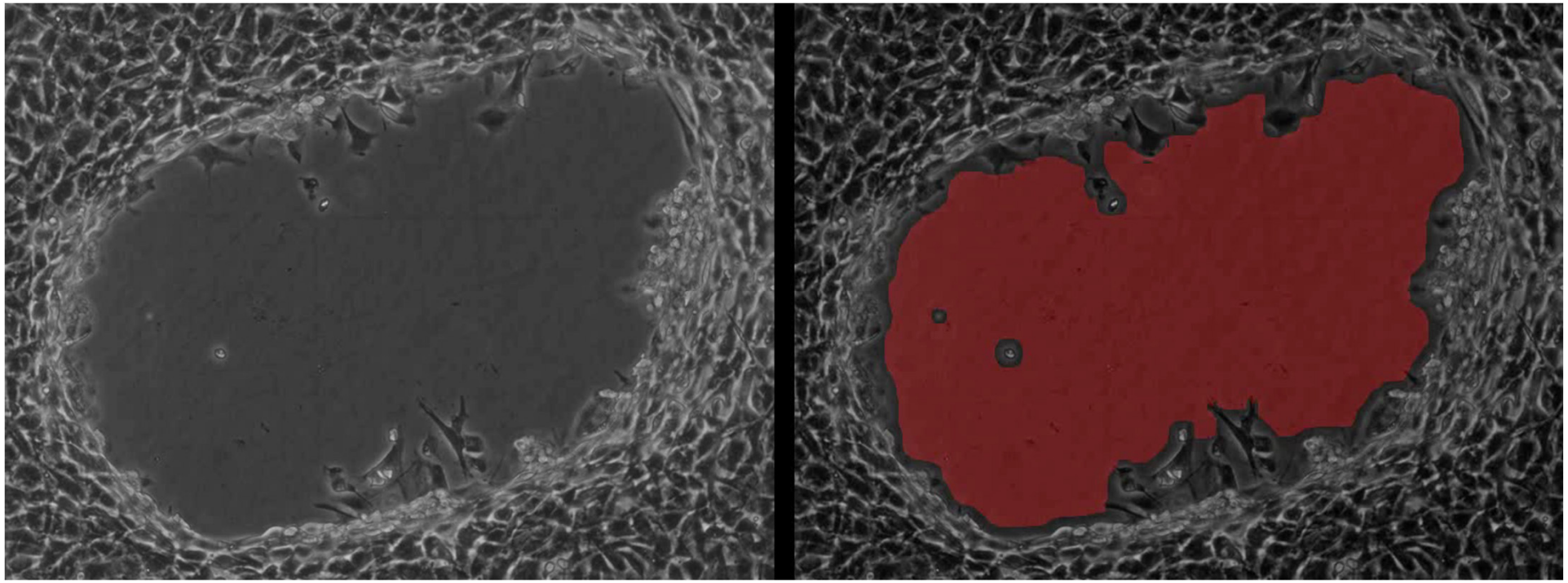




* The algorithm is generic and will function regardless of how damage has been inflicted

Topman et al. *Microscopy & Microanalysis*, 2011
Topman et al. *Med Eng Phys*, 2012

Measuring collective cell migration: Automated assays (I)

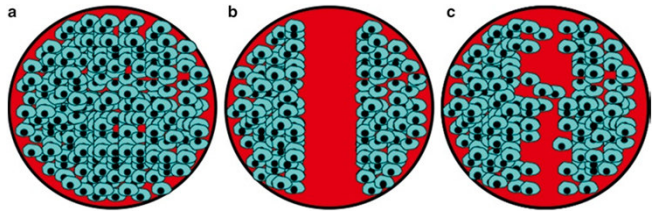


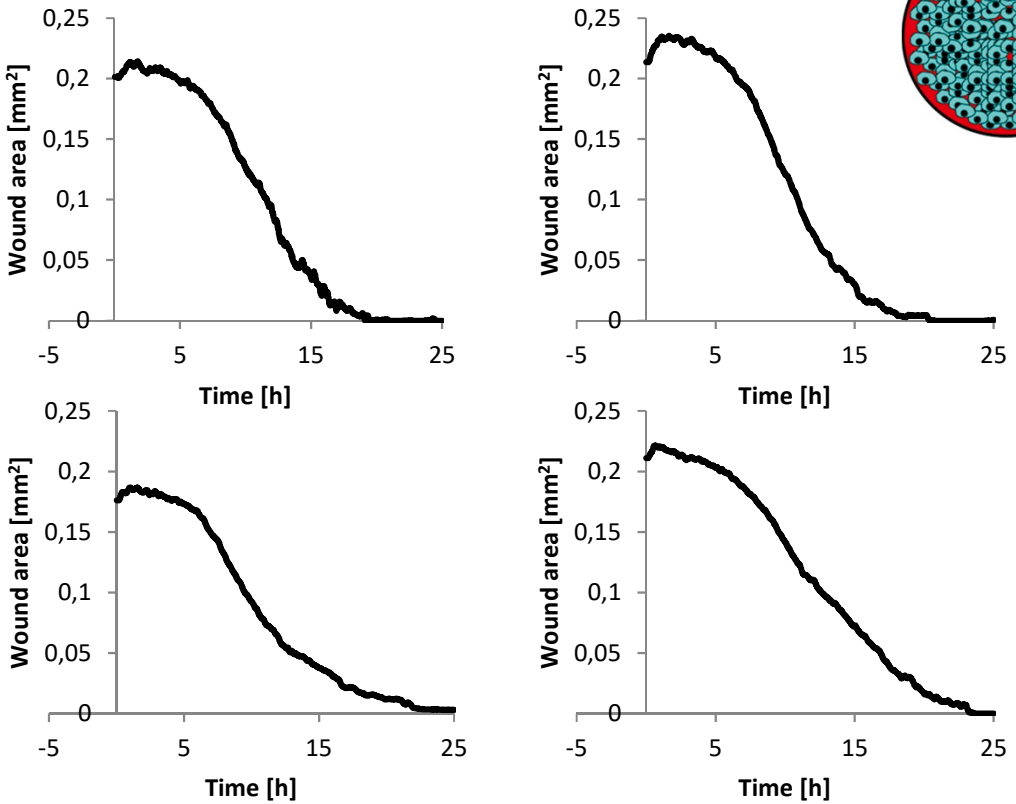
Analysis over a time course

Topman et al. *Med Eng Phys*, 2012

Measuring collective cell migration: Automated assays (II)

Example wound area versus time plots:

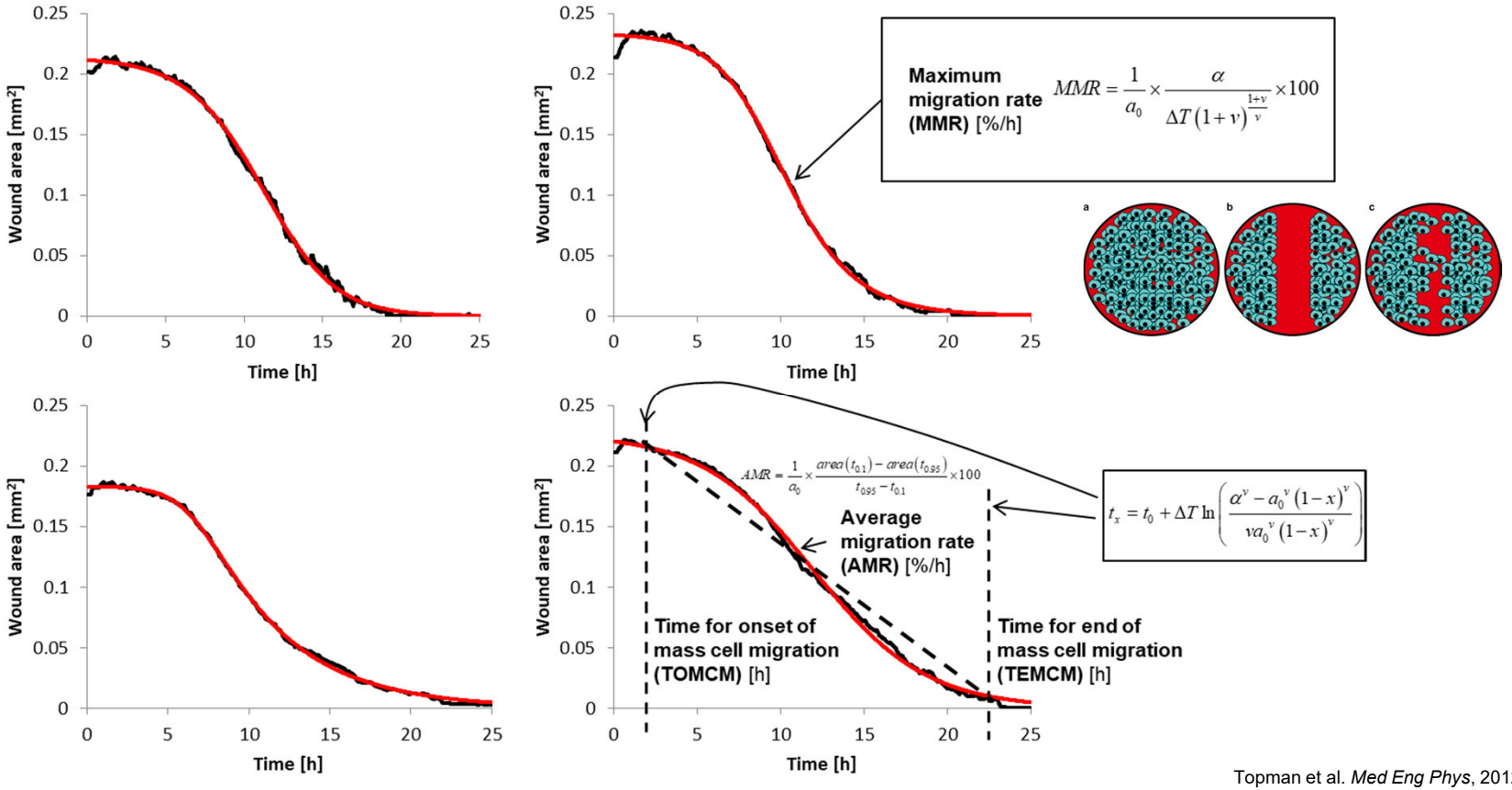




Topman et al. *Med Eng Phys*, 2012

Measuring collective cell migration: Automated assays (III)

Automated fitting of Richard's function:



Topman et al. *Med Eng Phys*, 2012

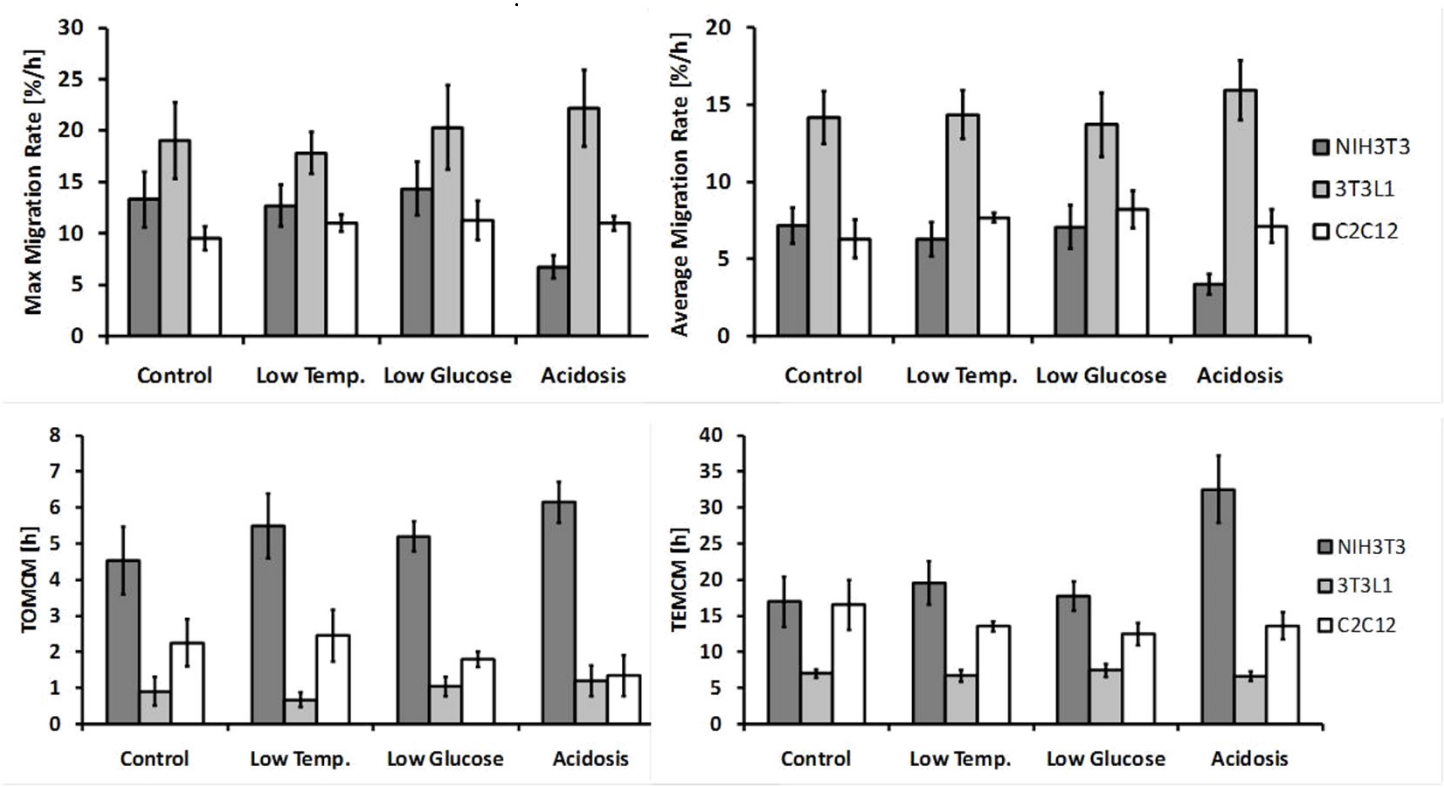
Effects of simulated exudate conditions on collective migration (I)

Condition / Factor	Control	Low Glucose	Low Temperature	Acidosis
Glucose [g/l]	4.5	1.0	4.5	4.5
Temperature [^{0}C]	37	37	35	37
pH	7.6	7.6	7.6	6.7

* Migration rate measurements were repeated 6 times (in different cultures) per each experimental condition

Topman et al. *Annals of Biomedical Engineering*, 2012

Effects of simulated exudate conditions on collective migration (II)

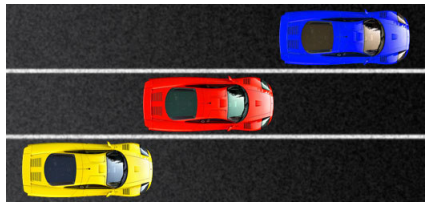


* TOMCM= time of onset of mass cell migration; TEMCM= time of end of mass cell migration

Topman et al. *Annals of Biomedical Engineering*, 2012

Effects of simulated exudate conditions on collective migration (III)

- Different cell types migrate at different velocities – some are faster than others



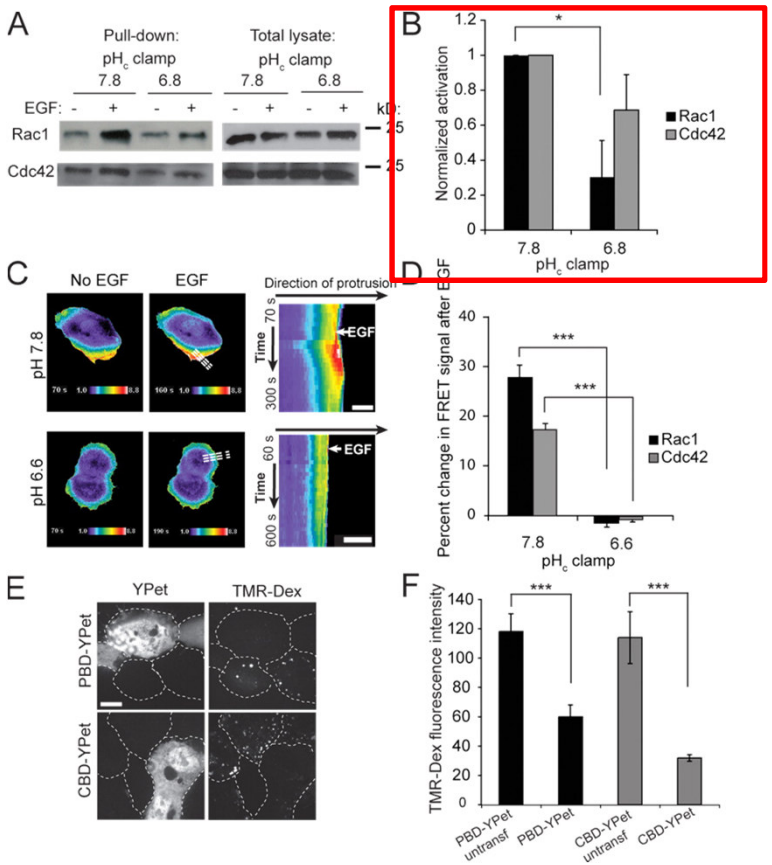
- Acidosis hindered migration of NIH3T3 fibroblasts in monolayers

- *Clinical relevance*:

  Aerobic bacteria lower exudate pH, reducing cell motility & slowing healing

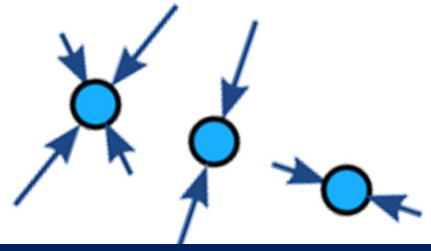
Topman et al. *Annals of Biomedical Engineering*, 2012

Biological mechanisms for the suppressing effect of low pH on migration



There is experimental evidence that Rac/Cdc42 activation – essential for lamellipodia formation – is impaired by decreased cytosolic pH

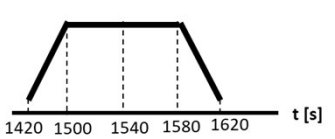
Koivusalo M, Welch C, Hayashi H, Scott CC, Kim M, Alexander T, Touret N, Hahn KM, Grinstein S.
Amiloride inhibits macropinocytosis by lowering submembranous pH and preventing Rac1 and Cdc42 signaling. J Cell Biol. 2010;188(4):547-63

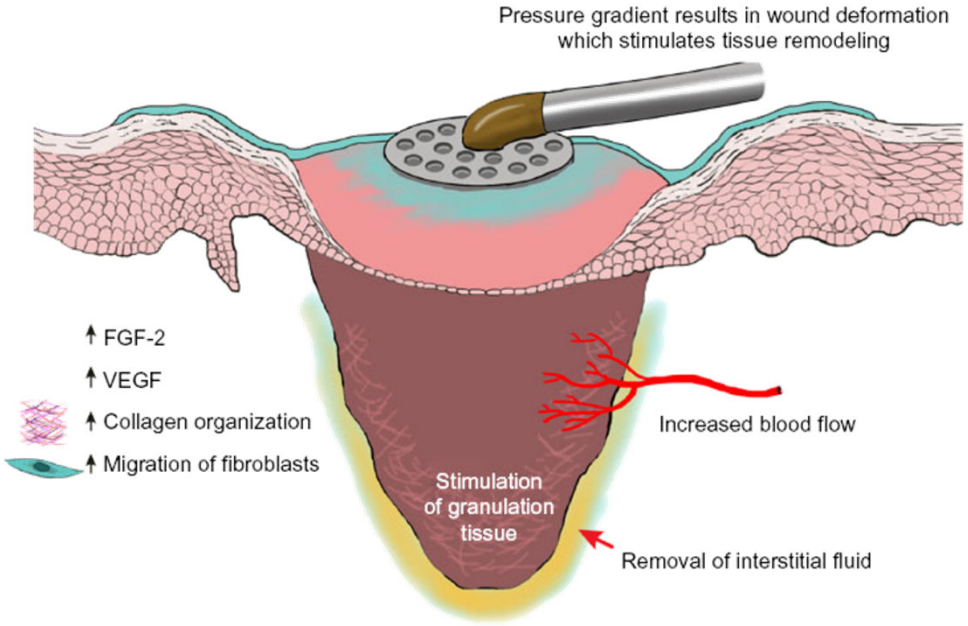


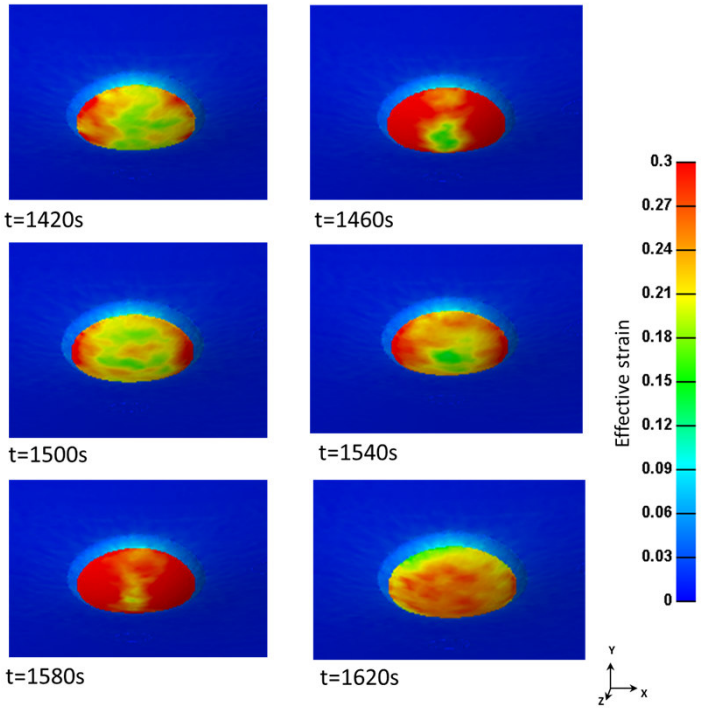
Studies of the impact of the mechanical environment of the wound bed (combined with exudate conditions) on collective migration

The mechanical environment induced in tissues by NPWT systems

Finite element simulations of the effects of pressure wave shapes on tissue deformations

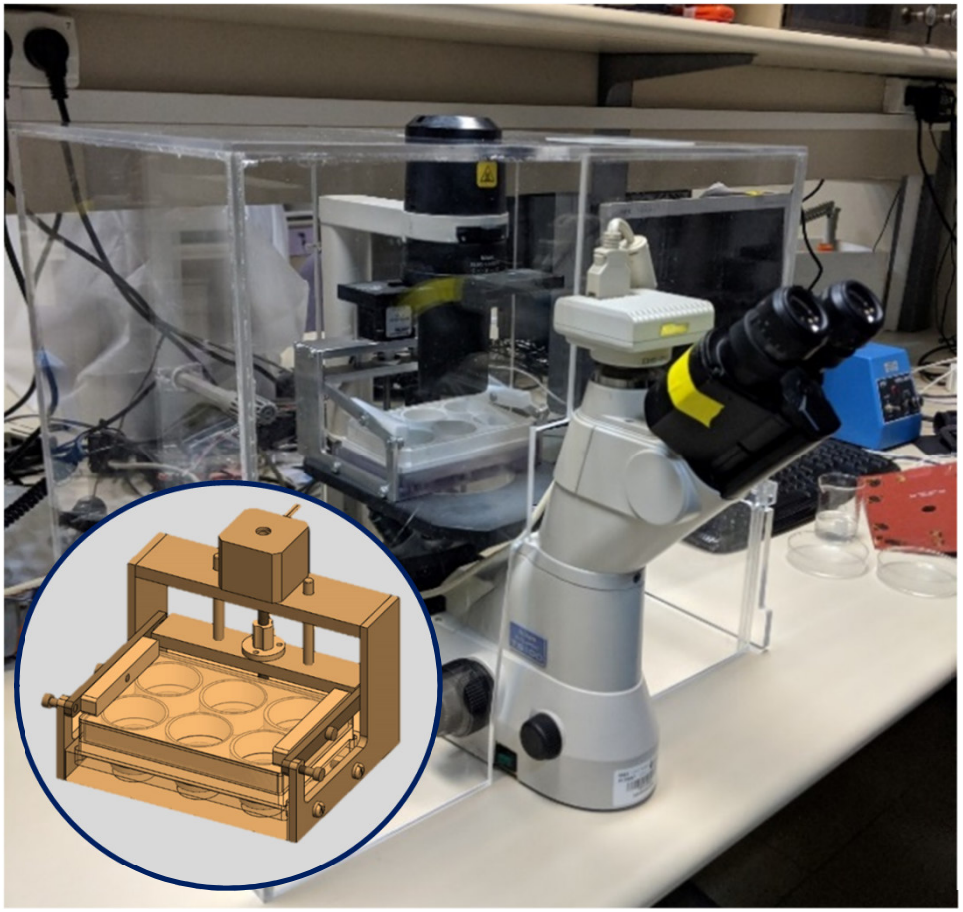


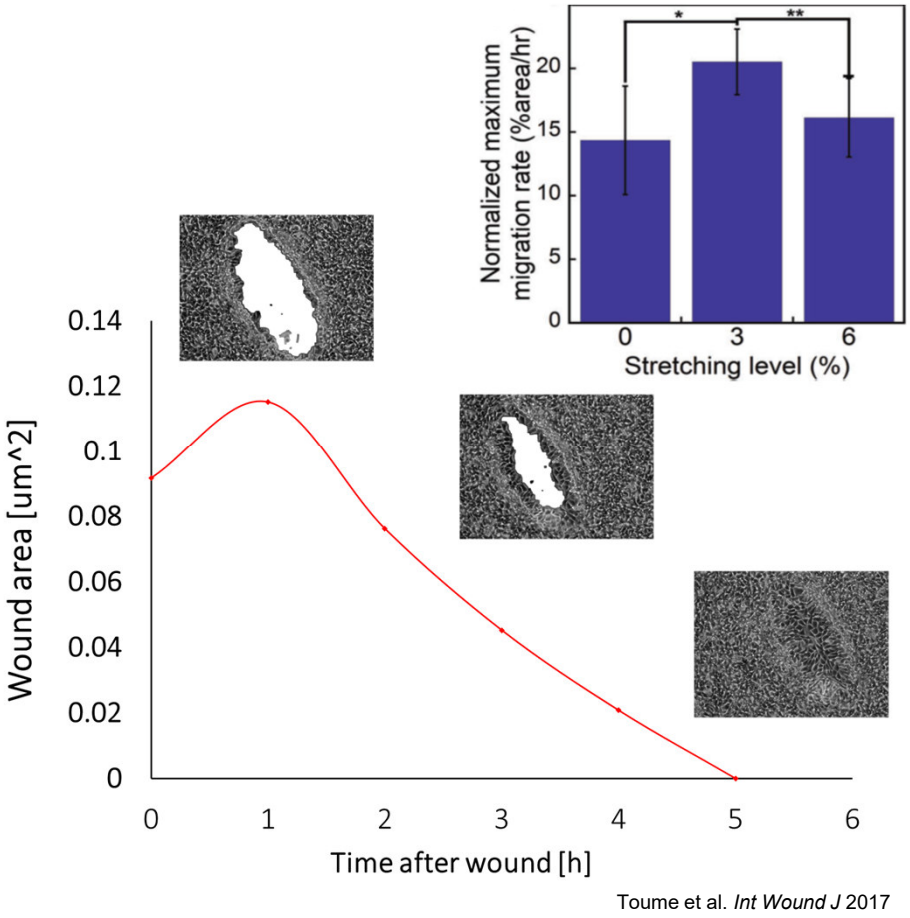




Katzengold, Gefen et al. *Int Wound J* 2017

Simulating NPWT-induced mechanical environments at the cell level

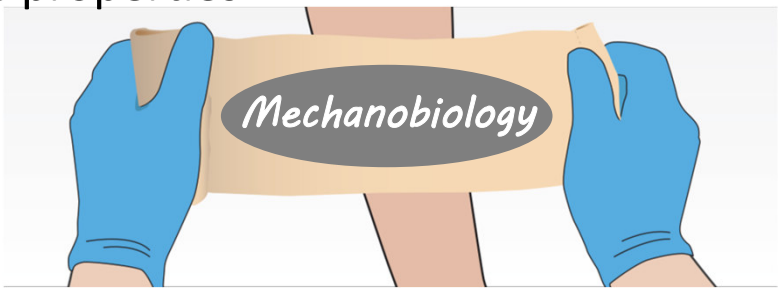




Toume et al. *Int Wound J* 2017

Discussion & conclusions

- Wound exudate should not be seen as merely a clinical management problem
- The exudate composition and properties influence migration of tissue-repairing cells
- The mechanical environment of the wound bed also affects proliferation & migration
- Mechanobiology indicates which specific exudate properties are conducive to healing
- Future dressings may actively tune these exudate properties



Collaborators and graduate students over the years

Collaborators

Prof. Dan Bader	University of Southampton
Prof. Joyce Black	University of Omaha
Prof. David Brienza	University of Pittsburgh
Prof. Mandy Fader	University of Southampton
Prof. Susan Margulies	Georgia Institute of Technology
Dr. Jan Kottner	Charite Medical University Berlin
Prof. Jane Nixon	University of Leeds
Prof. Cees Oomens	Eindhoven University of Technology
Prof. Yohan Payan	Joseph Fourier University Grenoble
Prof. Nick Santamaria	The University of Melbourne
Dr. Nogah Shabshin	Hospital of the University of Pennsylvania
Prof. Fred Vermolen	Delft University of Technology
Prof. Daphne Weihs	Technion – Israel Institute of Technology
Prof. Fiona Coyer	Queensland University of Technology
Dr. Michelle Barakat-Johnson	University of Sydney

Graduate students

Dr. Eran Linder-Ganz
Dr. Jonathan Elsner
Dr. Sigal Portnoy
Dr. Noa Slomka
Dr. Naama Shoham
Dr. Ran Sopher

Mr. Gilad Yarnitzky
Ms. Limor Agam
Ms. Yael Ruschkewitz
Ms. Malka Shilo
Mr. Samer Toume
Mr. Eran Atlas
Mr. Gil Topman, deceased
Mr. Tal Zeevi

Ms. Efrat Leopold
Ms. Ayelet Levy
Ms. Rona Katzengold
Ms. Lea Peko-Cohen
Ms. Maayan Lustig
Ms. Dafna Schwartz
Ms. Golan Amrani
Ms. Adi Lustig