Biophysical Medicine:

Therapeutic Advances in Wound Care

Rick Isenberg, MD
November 21, 2008

Disclosure

Dr. Isenberg is an employee of Regenesis Biomedical, Inc.

Regenesis manufactures and distributes a biophysical device for wound care.

Biophysical Medicine

A *physical* approach to the study of *biological* processes

- Ranging in scale from the sub-molecular

  Studying the interplay of the inter-atomic forces that give proteins their particular shape, motion and function

- To the systems level

  Studying the concerted activity of neural and genetic circuits.

University of California, Berkley, Dept of Biophysics

Biophysical Modalities in Wound Healing

- Mechanical
  - Negative Pressure Wound Therapy (NPWT)
  - Low Frequency Ultrasound
  - Extracorporeal Shockwave
- Electromagnetic
  - Pulsed Radio Frequency Energy (PRFE)

Negative Pressure Wound Therapy

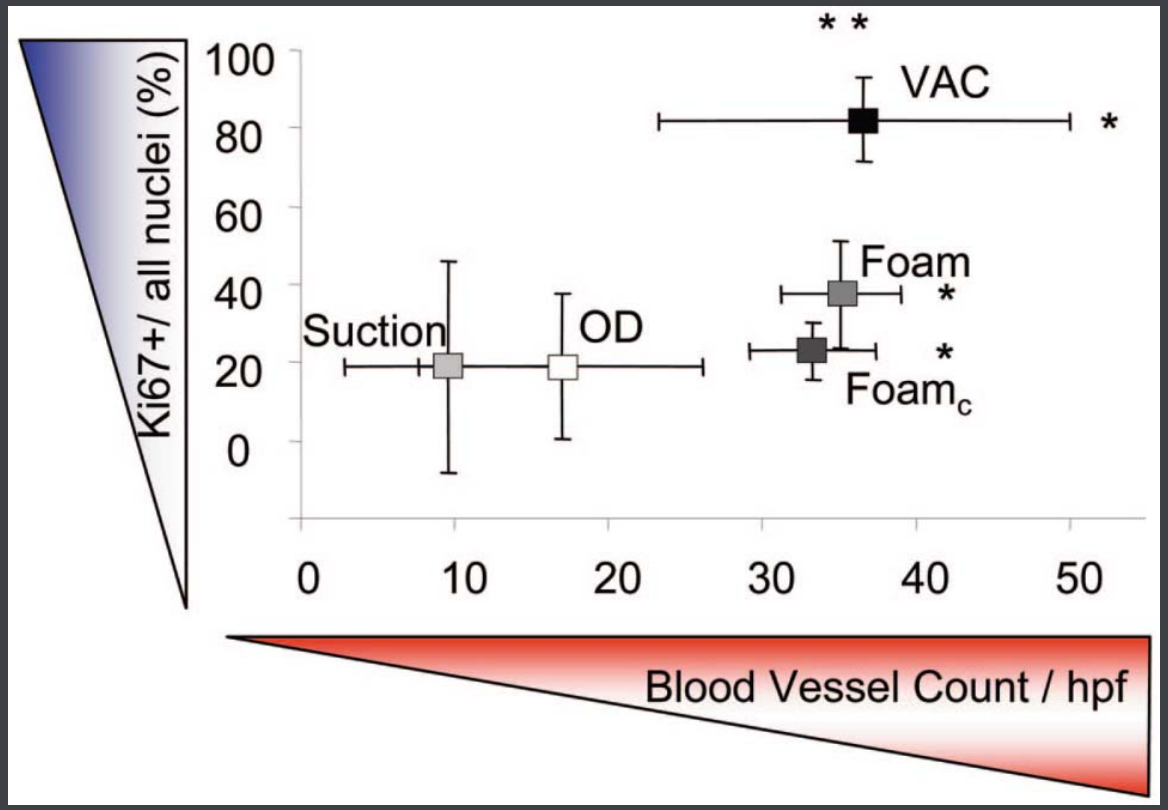
Negative Pressure Wound Therapy

Proposed Mechanisms of Action

- Removes excess interstitial fluid
- Decreases bacterial colonization
- Increases vascularity
- Stimulates granulation tissue formation through micromechanical deformations

Negative Pressure Wound Therapy

Blood Vessel Density and Proliferation in Response to NPWT and its individual components

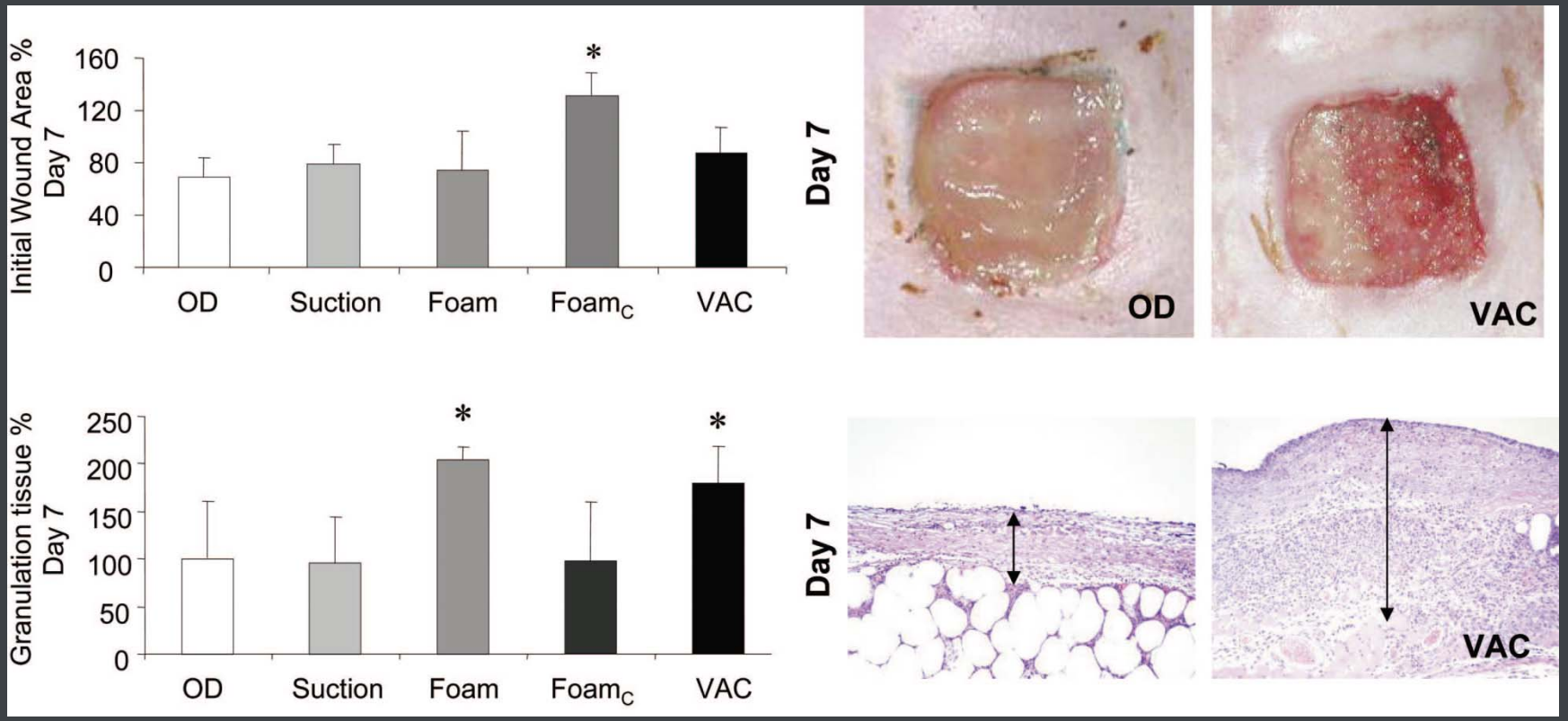


1. Polyurethrane foam appears to enhance blood vessel density
2. Foam with occlusive dressing and suction (VAC) induced cell proliferation significantly more than the individual components

Scherer SS. Plast Reconstr Surg 122: 786-797, 2008

Negative Pressure Wound Therapy

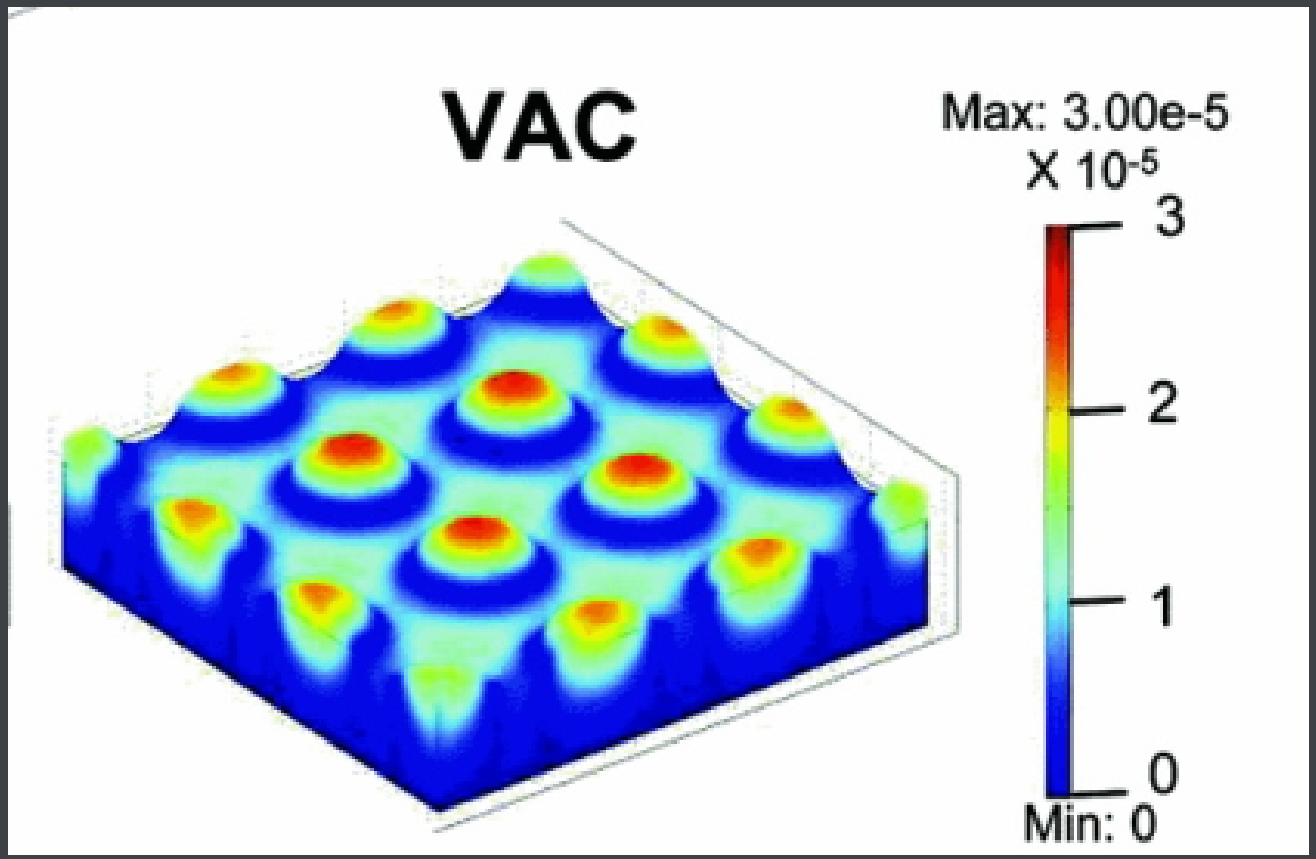
Granulation response to NPWT



Scherer SS. Plast Reconstr Surg 122: 786-797, 2008

Negative Pressure Wound Therapy

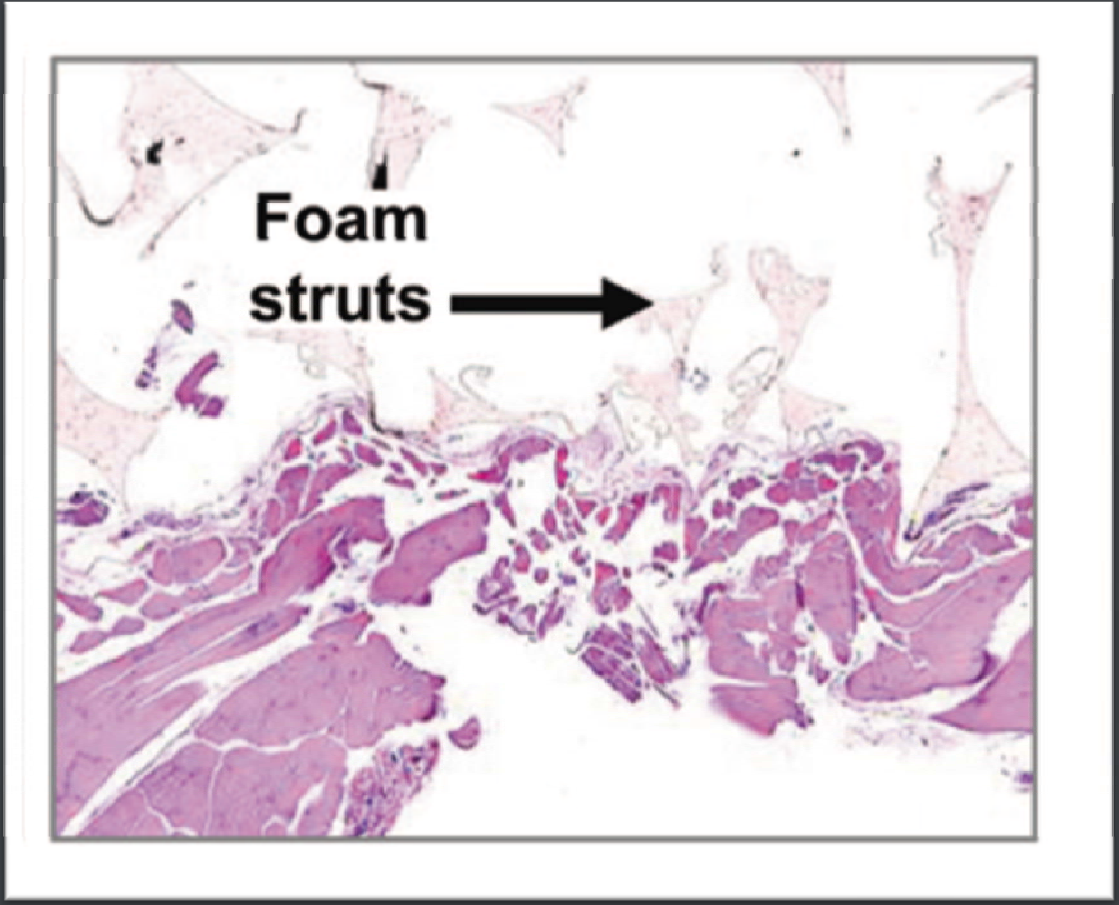
Mathematical model of tissue stress distribution with NPWT.



Scherer SS. Plast Reconstr Surg 122: 786-797, 2008

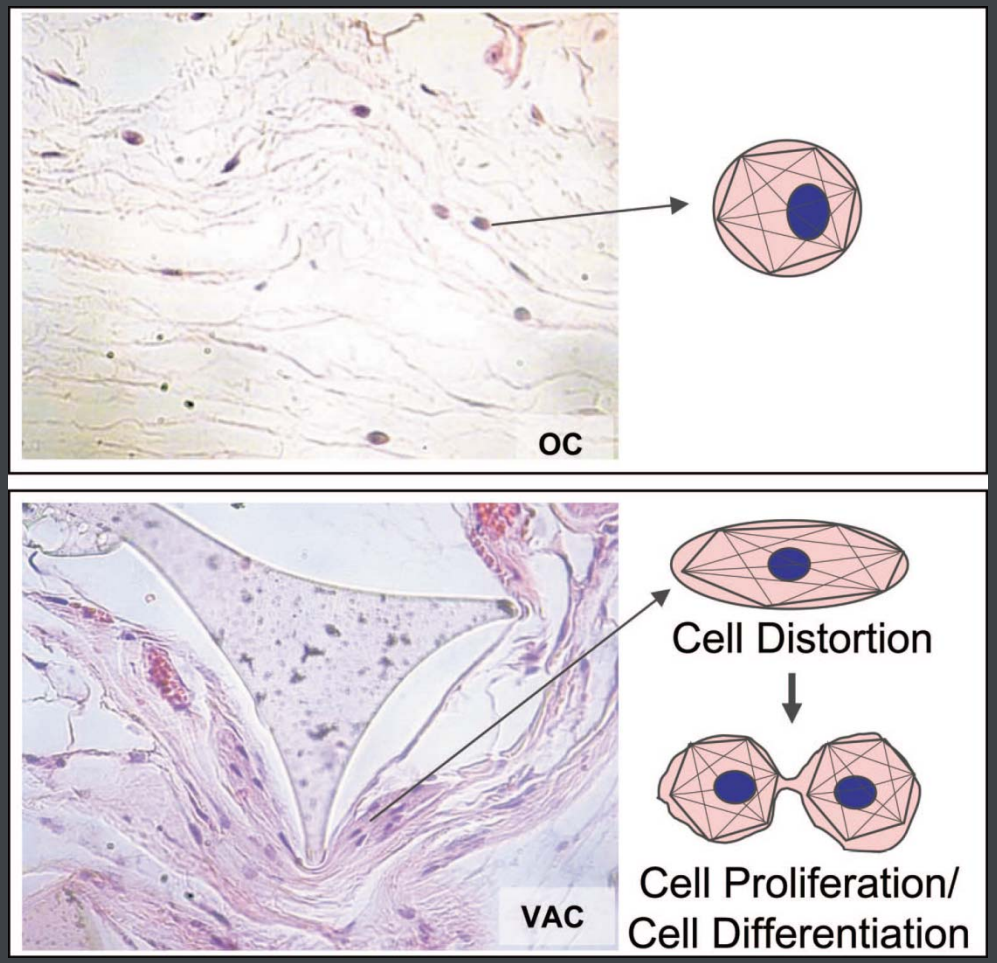
Negative Pressure Wound Therapy

Tissue Deformation with NPWT



Scherer SS. Plast Reconstr Surg 122: 786-797, 2008

Negative Pressure Wound Therapy



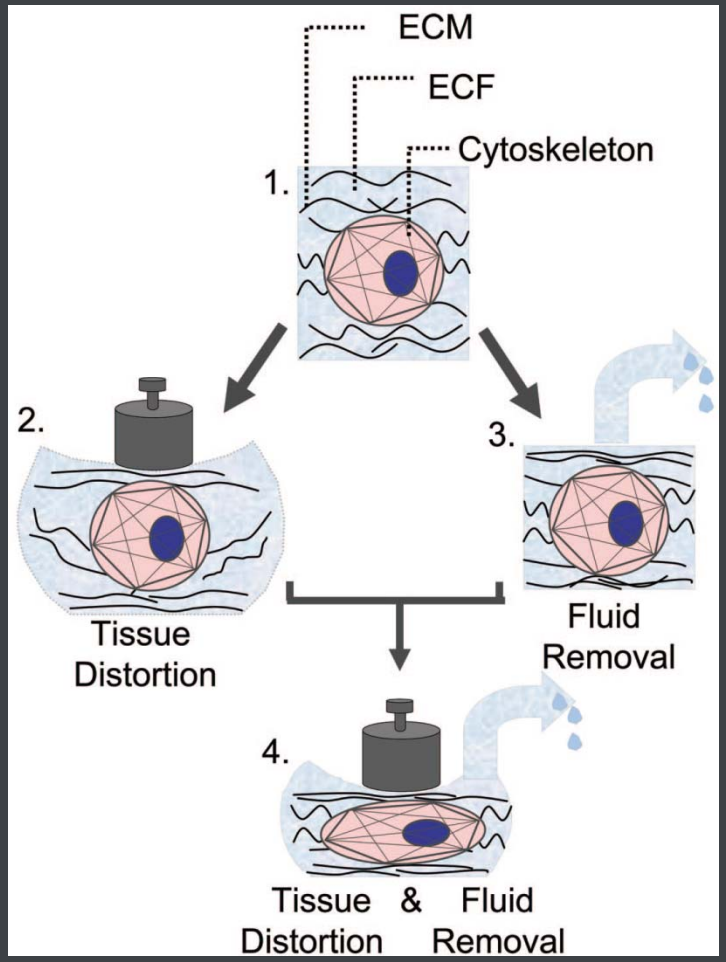
Cell distortion

Cells exposed to vacuum-assisted closure therapy showed mechanical deformation that was not seen with the other treatment modalities.

Distortion of the cytoskeleton can lead to cell proliferation through mechano-transduction.

Scherer SS. Plast Reconstr Surg 122: 786-797, 2008

Negative Pressure Wound Therapy



Fluid and mechano-transduction.

1. Tissues consist of cells embedded and attached to an extracellular matrix (ECM).
2. Tissue distortion starts to deform the incompressible extracellular fluid (ECF) and decoils structures of the extracellular matrix without affecting the shape of the single cells.
3. Fluid removal concentrates the components of the extracellular matrix in the tridimensional space but does not affect the shape of the single cells.
4. Forces acting on tissues with reduced extracellular fluid affect single-cell morphology by distortion.

Scherer SS. Plast Reconstr Surg 122: 786-797, 2008

Non-Contact Low Frequency Ultrasound

Therapeutic Ultrasound

	High Intensity Ultrasound	Low Intensity Ultrasound
High Frequency (MHz)	Thermal Sports Medicine Physical Therapy	Diagnostic Imaging Fetal Monitoring
Low Frequency (KHz)	Debridement Söring, Misonix	**Wound Healing** Celleration

Low Frequency Ultrasound

Proposed Mechanisms of Action

- Enhanced angiogenesis
- Reduction of Bioburden

Mechanical Interaction

- Absorption
- Cavitation
- Acoustical Streaming

Low Frequency Ultrasound

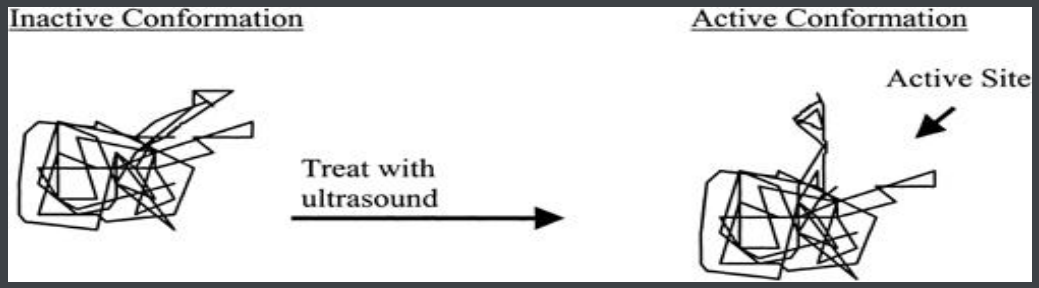
Cellular and Molecular Effects of Nonthermal Ultrasound on Wound Healing

Increase in Protein or Cellular Function	Producing Cell Type	Effector Function
Interleukin-1β[45]	Osteoblasts, monocytes	General inflammatory mediator
Interleukin-2[48]	T cells	T-cell growth
Interleukin-8[45,50]	Osteoblasts	Endothelial cell migration and proliferation
Vascular endothelial growth factor[45,50]	Osteoblasts, monocytes	Endothelial cell migration and proliferation
Basic fibroblast growth factor[45]	Osteoblasts	Endothelial cell migration and proliferation
Fibroblast growth factor[50,53]	Monocytes	Fibroblast growth
Collagen[5,45]	Osteoblasts, fibroblasts	Wound healing
Chloramphenicol acetyl transferase[56]	HeLa, NIH/3T3, C1271	Gene expression of liposomal transfection
Increased proliferation[45]	Fibroblasts	Enhanced wound healing
Increased proliferation[45]	Osteoblasts	Enhanced wound healing
Lymphocyte adhesion[41]	Endothelial cells	Enhanced lymphocyte trafficking
Vasodilation[39,40,42]	Capillary, endothelium	Enhanced blood flow

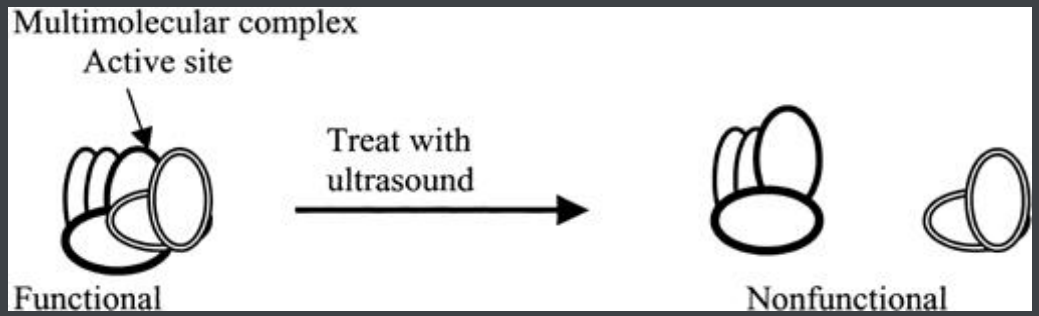
J Athl Train. 2002 Jul–Sep; 37(3): 293–299.

Low Frequency Ultrasound

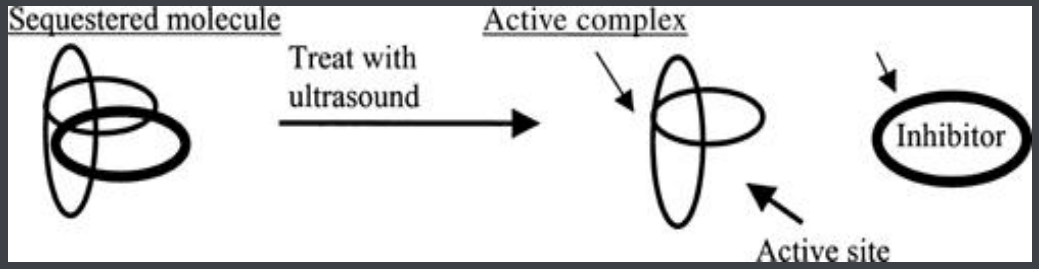
Frequency Resonance Hypothesis



Conformational Shift



Molecular Disjunction

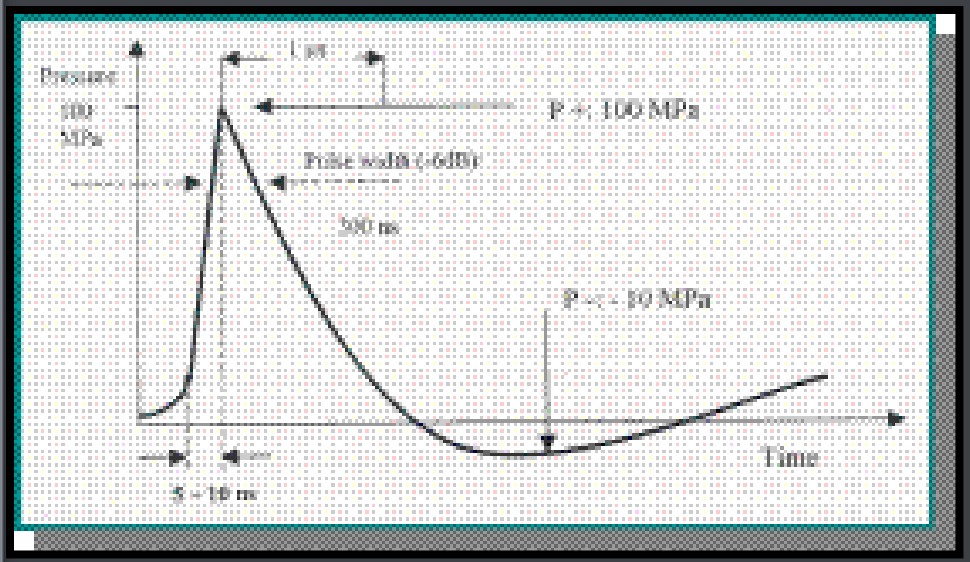


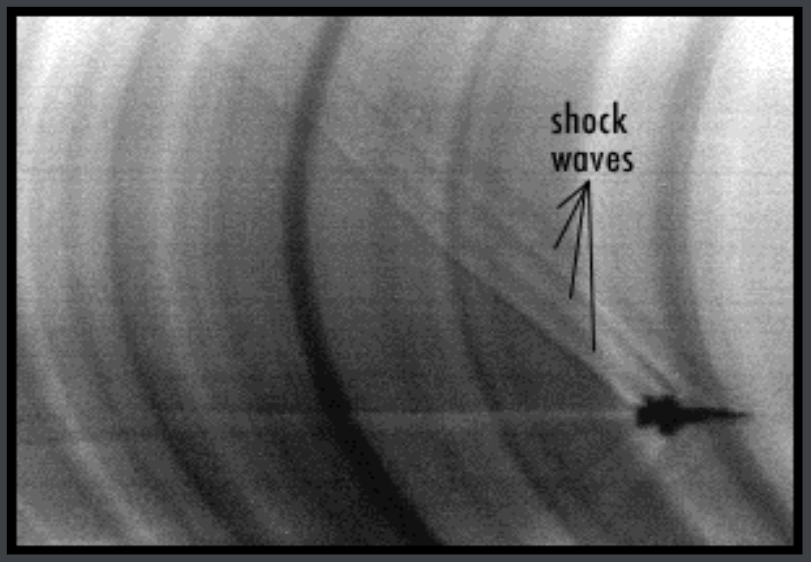
Dislodgement of Inhibitor

J Athl Train. 2002 Jul–Sep; 37(3): 293–299.

Extracorporeal Shock Wave Therapy

Extracorporeal Shock Wave





- Electrohydraulic acoustic pressure wave
- Multiple medical applications
  - Kidney stones
  - Plantar fasciitis
  - Nonunion fractures
  - Wound healing (?)

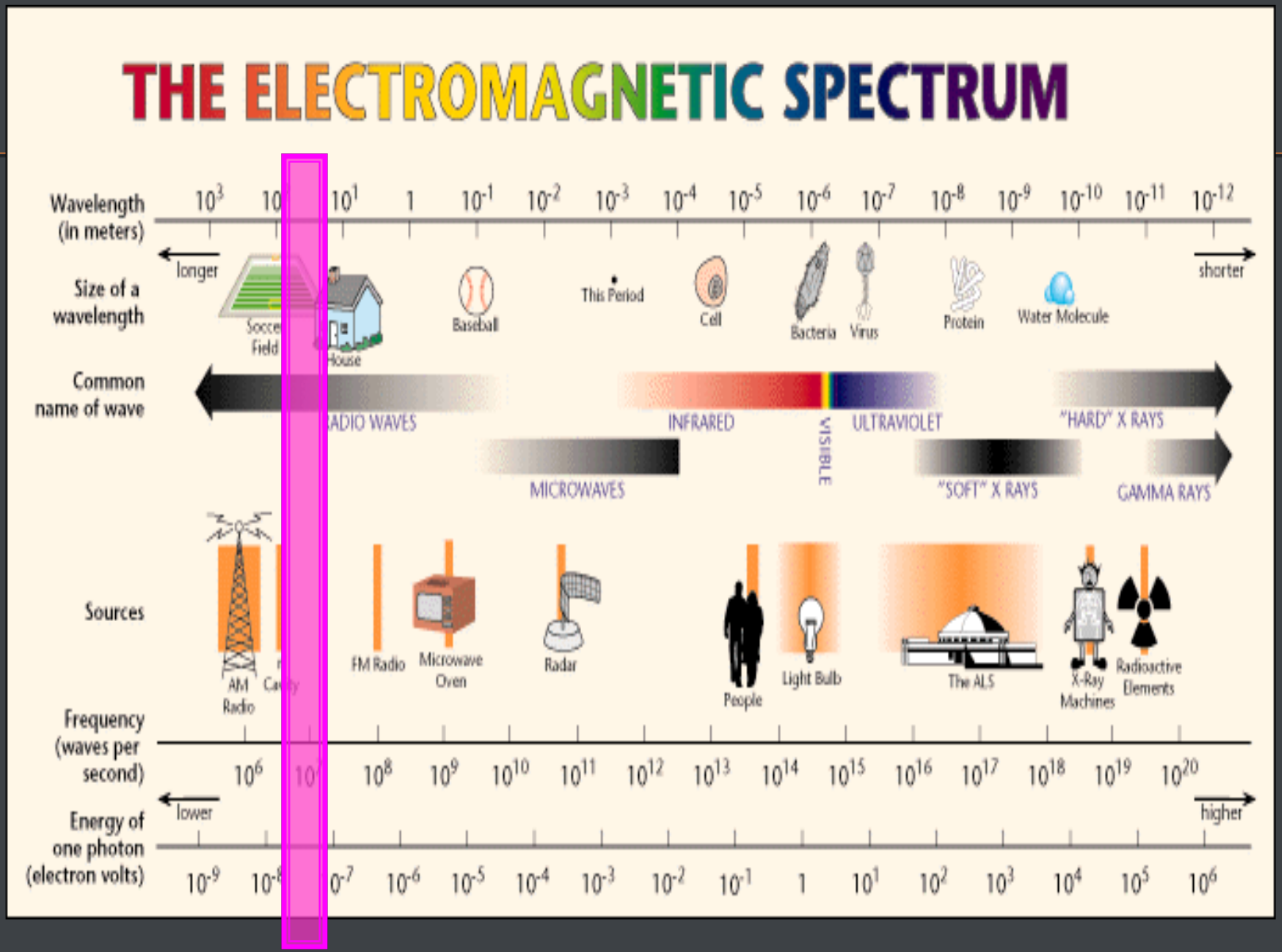
Extracorporeal Shock Wave

Proposed Mechanism of Action

- Enhanced revascularization
- Increased expression of pro-angiogenic chemokines, cytokines and matrix metalloproteinases
- Decreased expression of pro-inflammatory cytokines and recruitment of leukocytes

Stojadinovic et al. *Angiogenic response to extracorporeal shock wave treatment in murine skin isografts*. Angiogenesis. Epub 2008 Nov 9

Pulsed Radio Frequency Energy



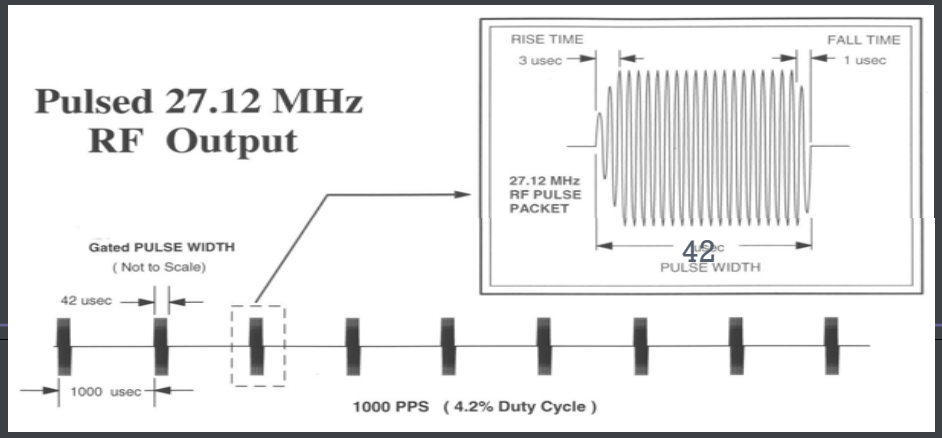
PRFE Signal

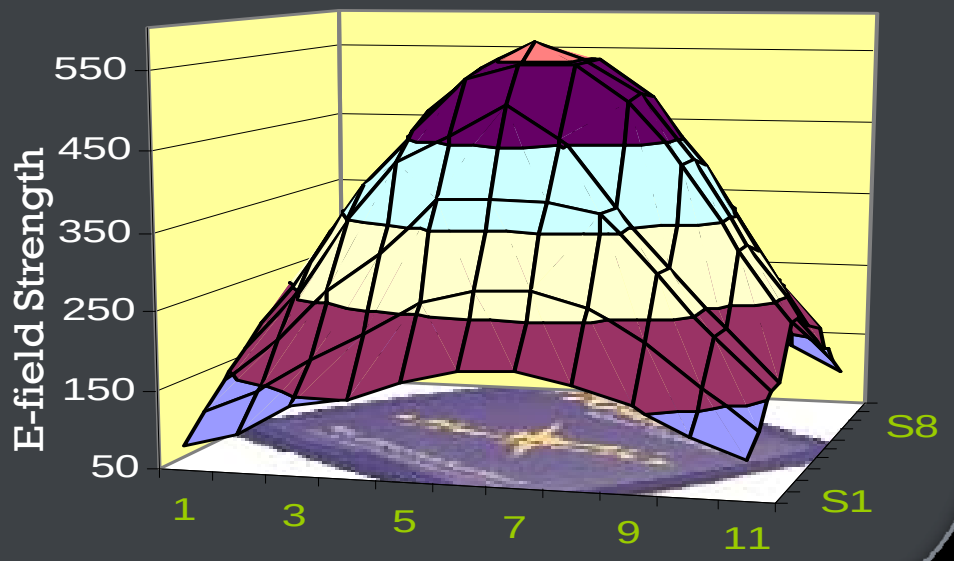
www.nih.gov

Pulsed Radio Frequency Energy

Pulsed Radio Frequency Energy (PRFE)

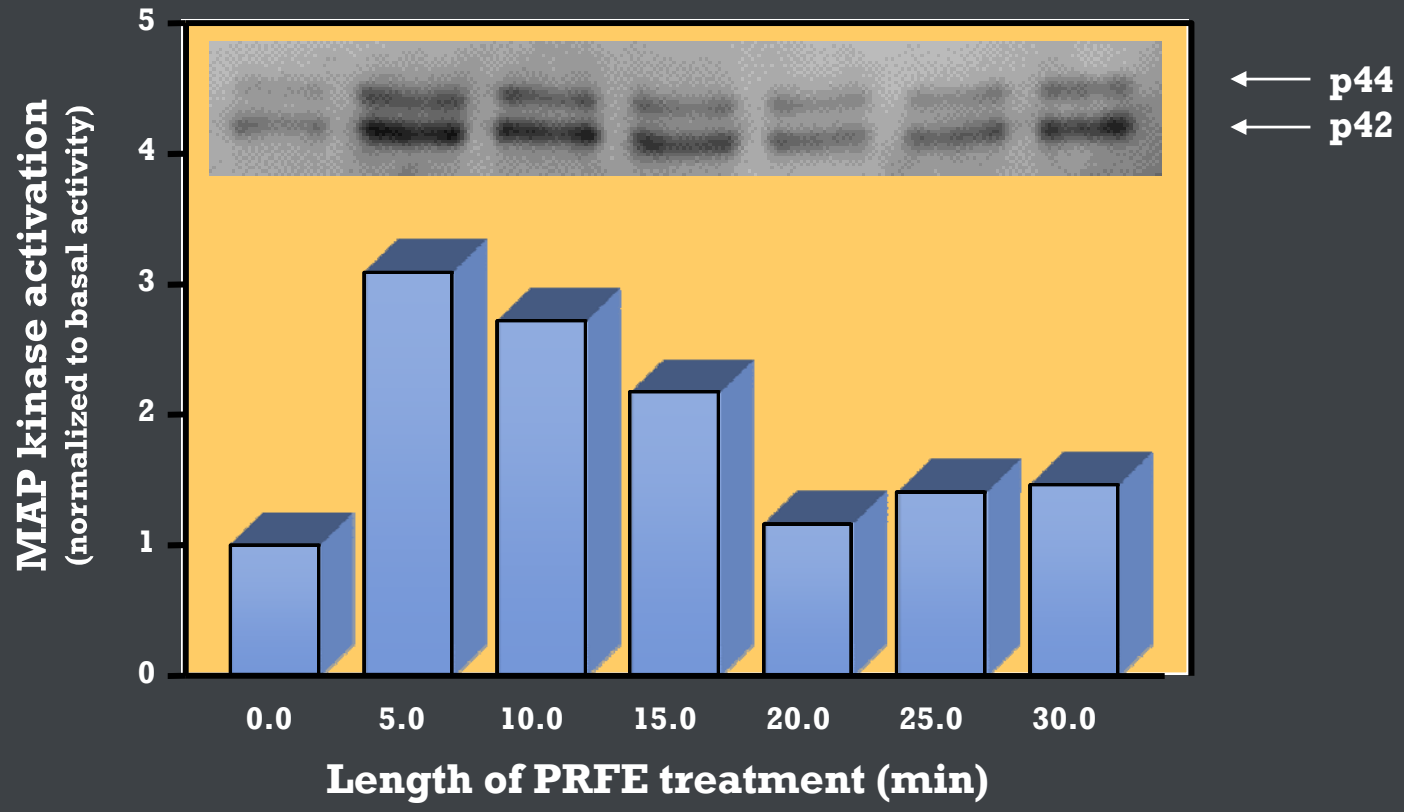
- **Radio frequency (27.2 MHz)**
- **Radio frequency field with optimized waveform parameters**
- **595 V/m field strength**
- **42 microsecond pulse**





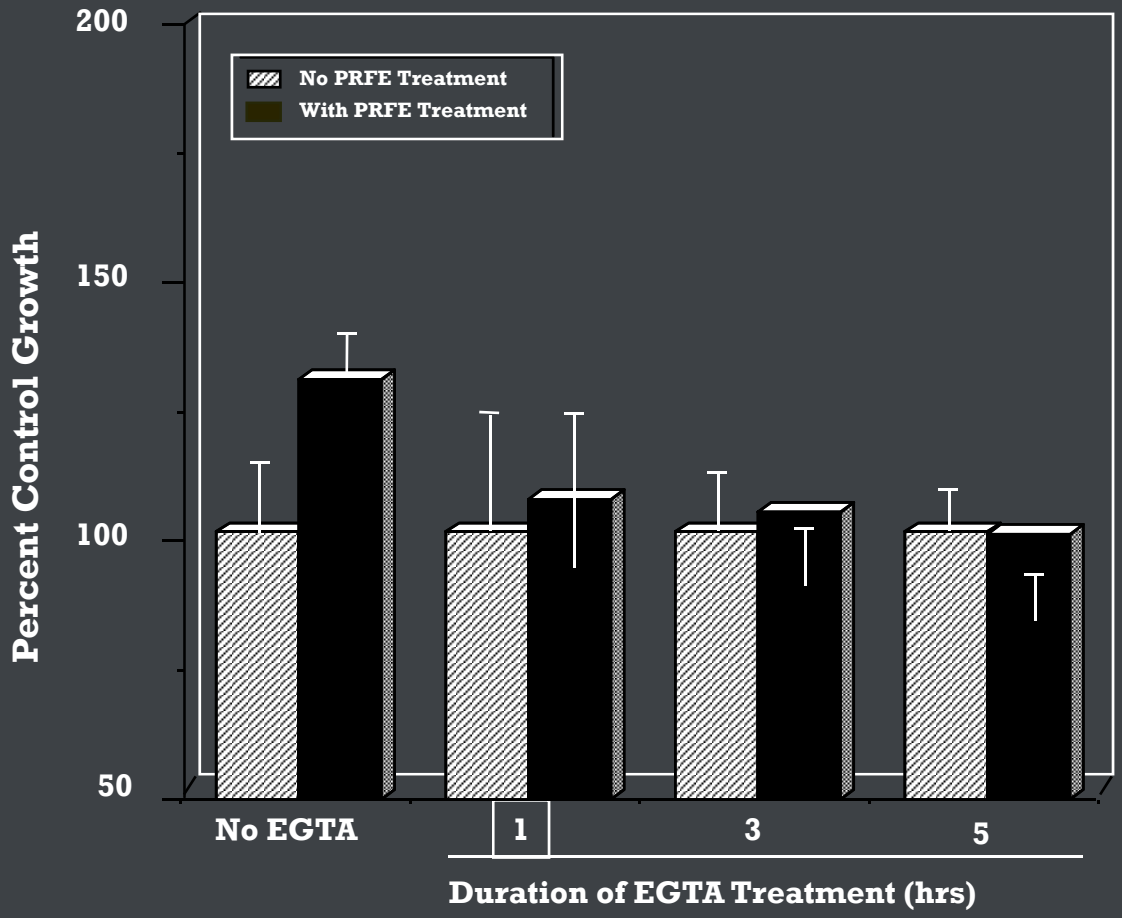
Pulsed Radio Frequency Energy

1. Rapidly activates MAP kinase cascade



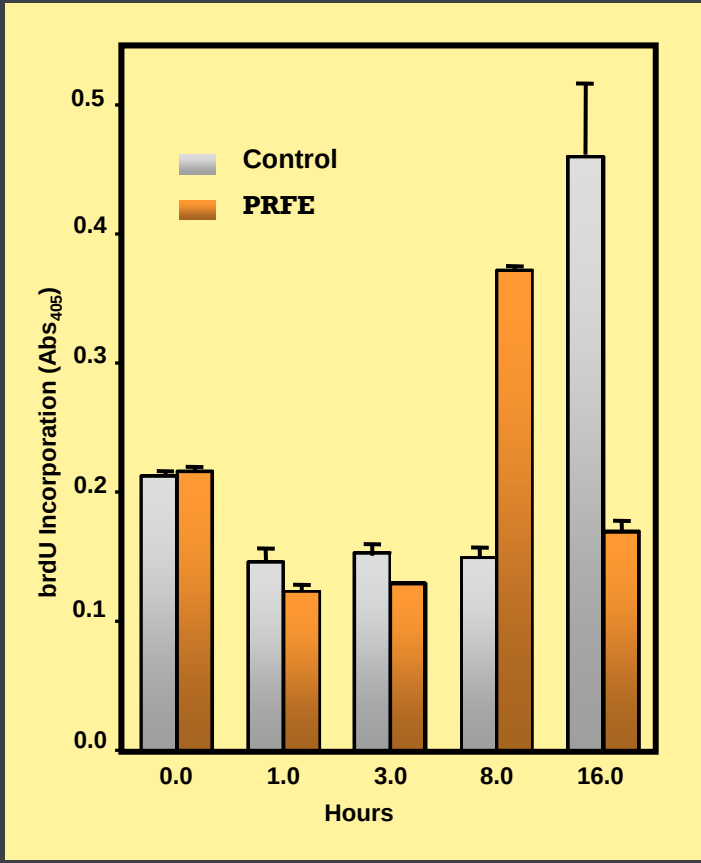
Pulsed Radio Frequency Energy

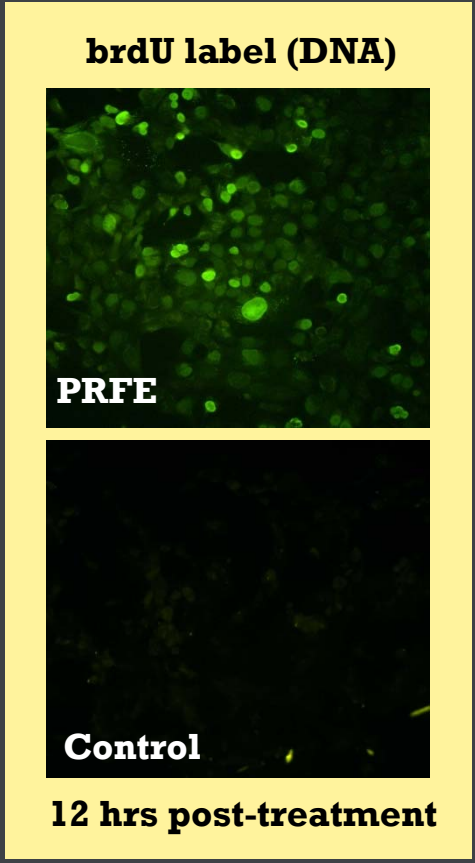
Calcium Mediated Effect



Pulsed Radio Frequency Energy

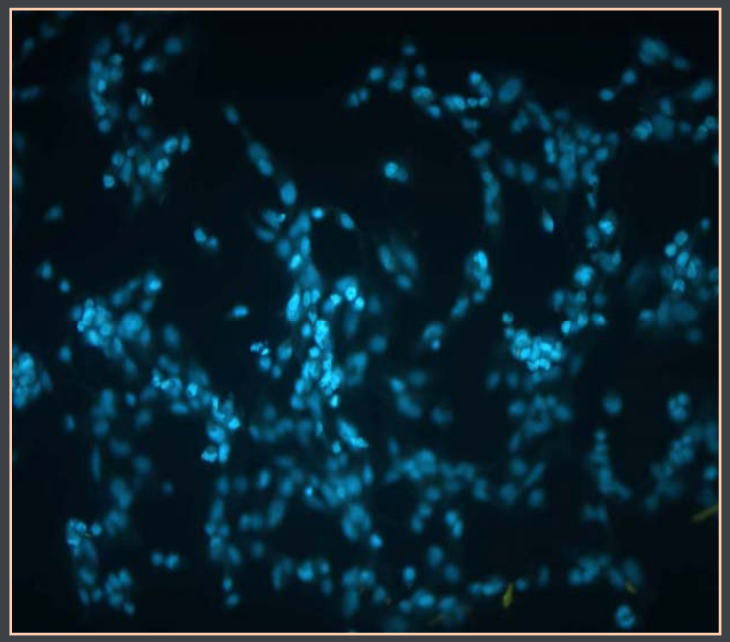
2. PRFE acceleratesDNA synthesis



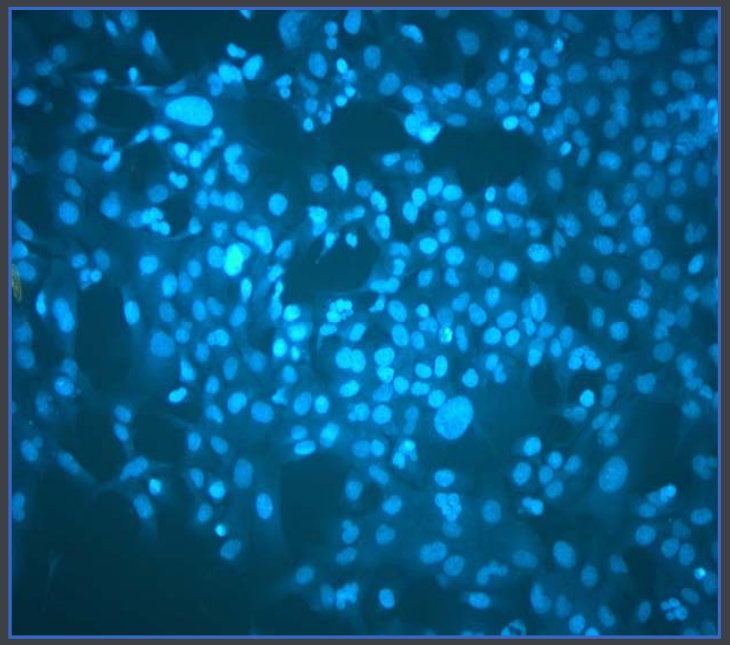


Pulsed Radio Frequency Energy

3. Stimulates cells critical to wound repair



Control

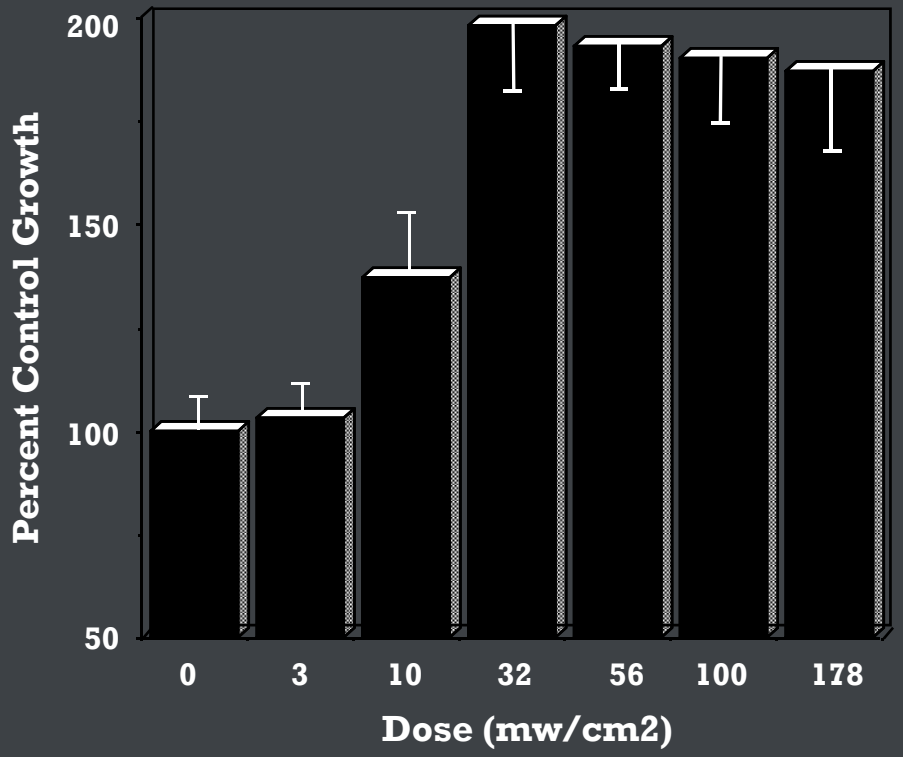


PRFE

12 hrs post-treatment, 100X, images of DAPI-stained Human Dermal Fibroblast (HDF) cell nuclei

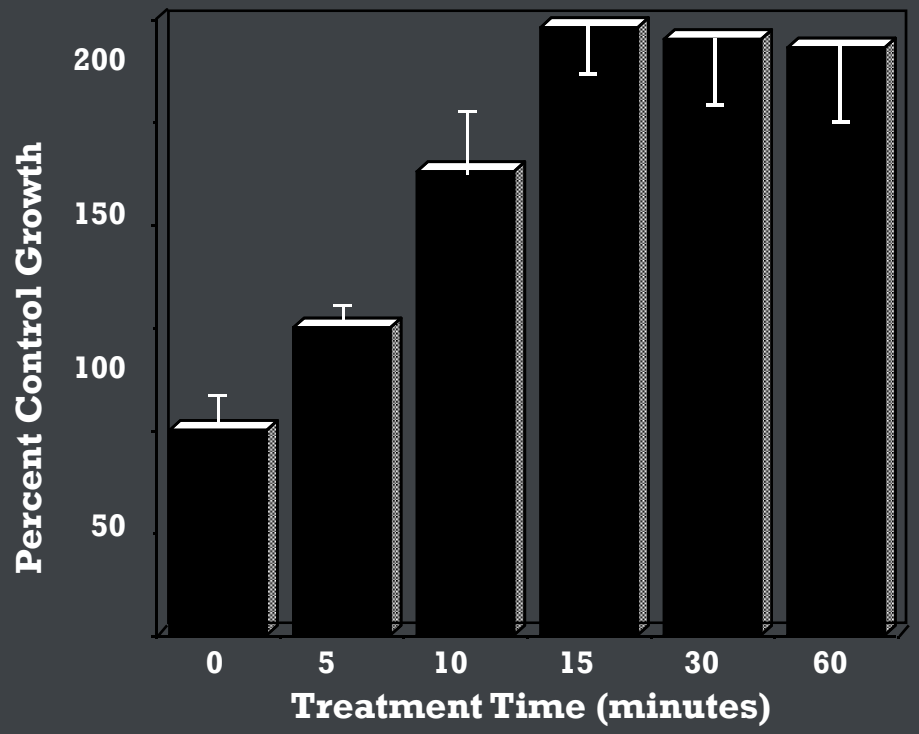
Pulsed Radio Frequency Energy

Dose Relationship



PRFE Effect on Cell Growth

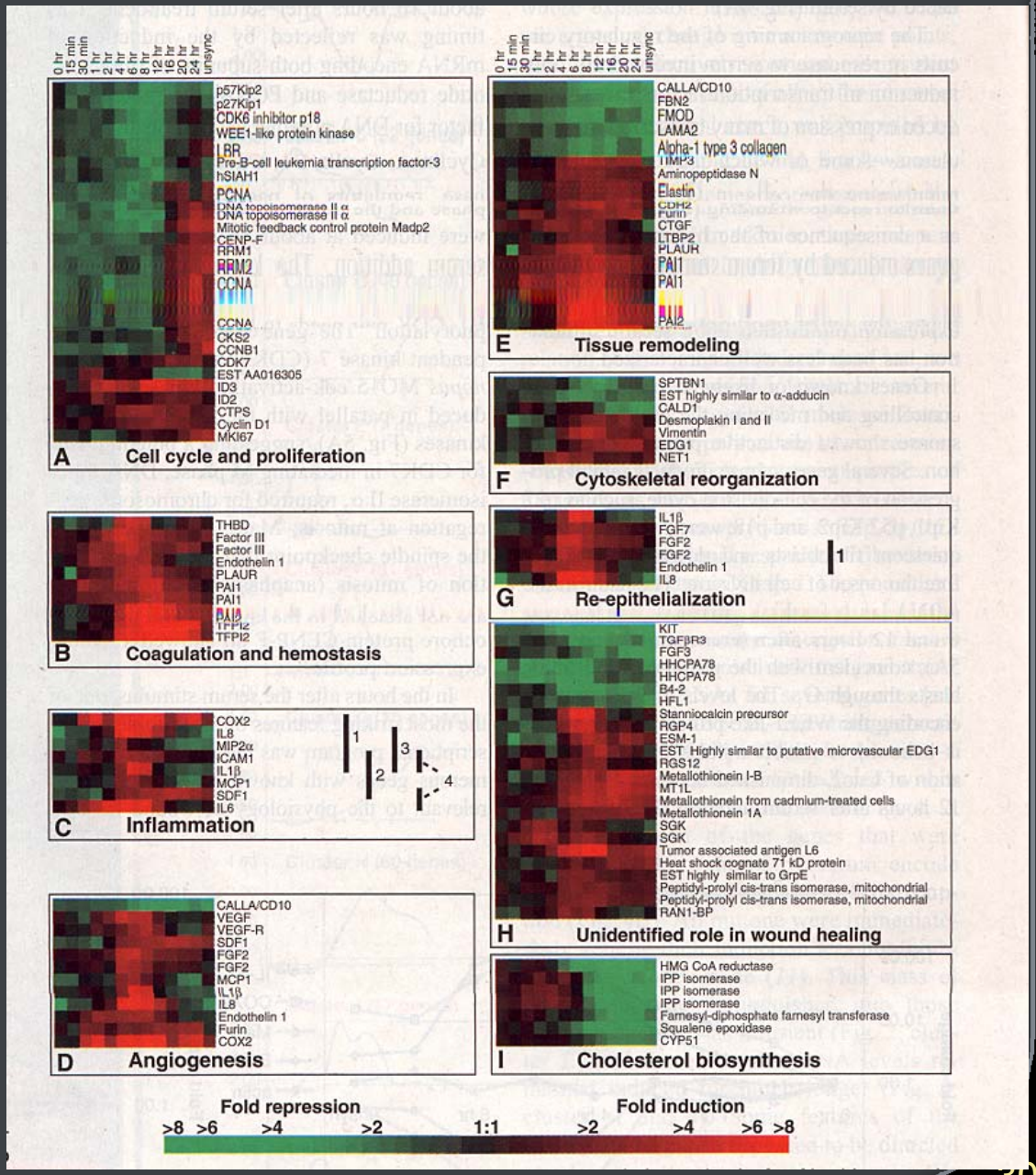
Treatment Time Relationship



What Initiates Wound Repair Sequence?

Using gene array assays, serum has been shown to be the prototypic wound repair signal (Iyer, et al., Science, 283:83-87,1999).

In this depiction of expression time frames for over 1,000 genes following exposure to serum, it can be seen that the "normal" timing for many repair and replication genes to "turn on" is at about 4-8 hours after the replication process is started.

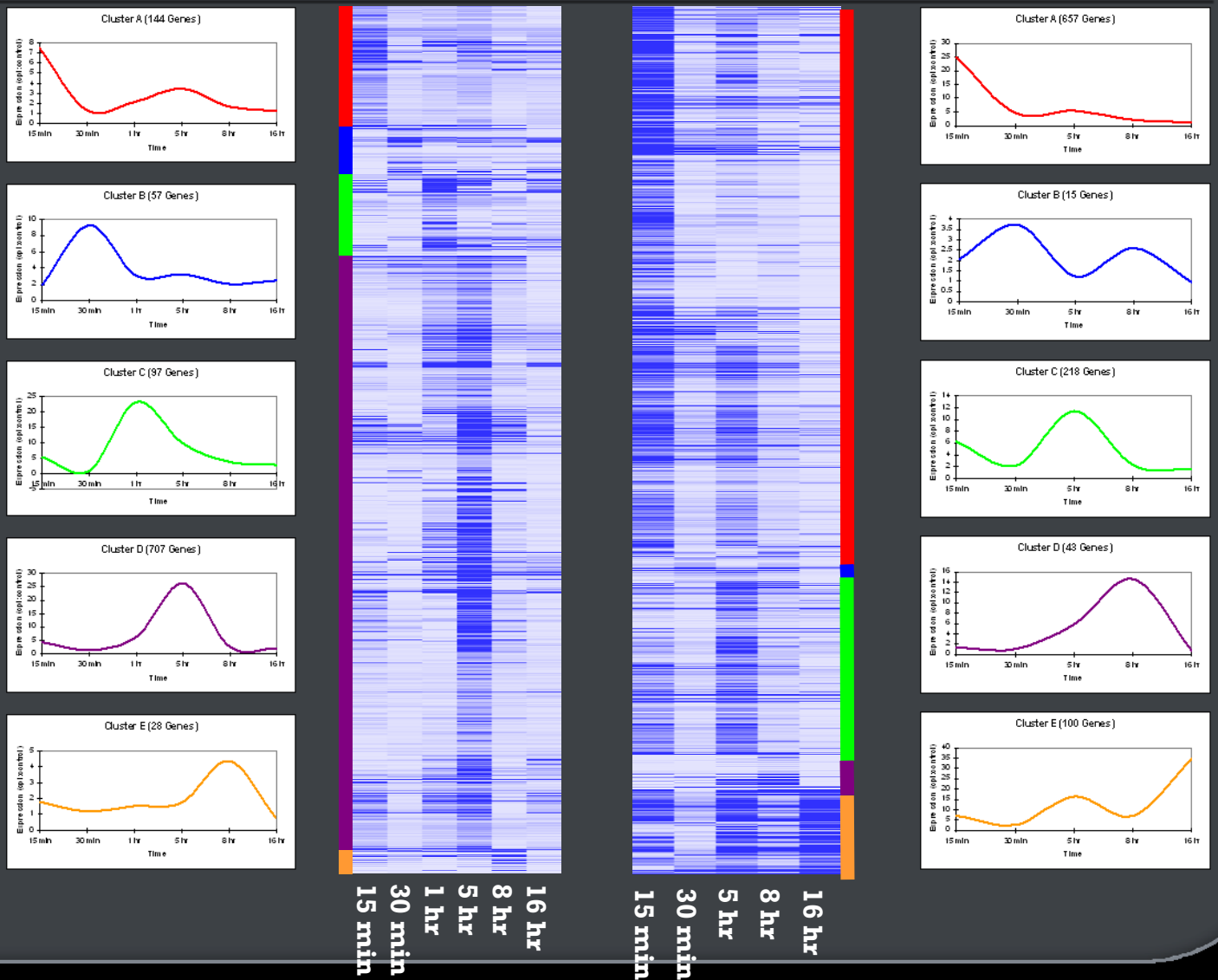


Pulsed Radio Frequency Energy

Serum Control

PRFE

Gene Expression Profiles



Pulsed Radio Frequency Energy

Examples of gene expression profiles regulated by both serum and PRFE

- **Signal transduction:** MAP Kinase P1, TGFBR3, VEGF
- **Transcription Factors:** c-fos, myc, EGR1, HSF 2
- **Cell Cycle:** Cyclin A1, D11, CDC2, DNA topoisomerase II
- **Inflammation:** TNFR1, IL1B
- **Angiogenesis:** CALLA/CD10, VEGF, IL1B, FGF2
- **Re-epithelialization:** IL1B, FGF2, FGF7, Endothelin 1
- **Tissue Remodeling:** CALLA/CD10, TIMP3

PRFE Proposed Mechanism

- Activates MAP Kinase cascade
- Accelerates DNA synthesis and triggers cellular proliferation
- Accelerates physiologic wound-healing genetic cascade
- Modulates gene transcription and production of growth factors, cyclins, cytokines

Biophysical Medicine: Summary

Energy, in its various forms, can:

- Alter the architecture and activity of cells, organelles and bioactive molecules
- Incite physiological and genetic responses which impacts the inflammatory and granulation phases of wound healing
- Allows for the development of non-invasive wound therapies

Contact

Rick Isenberg, MD

Email: isenberg@regenesisbiomedical.com

Phone: 480/970-4970