Invasive/Non-invasive Ultrasound Effects on Adipocytes and Adipose-Derived Stem Cells

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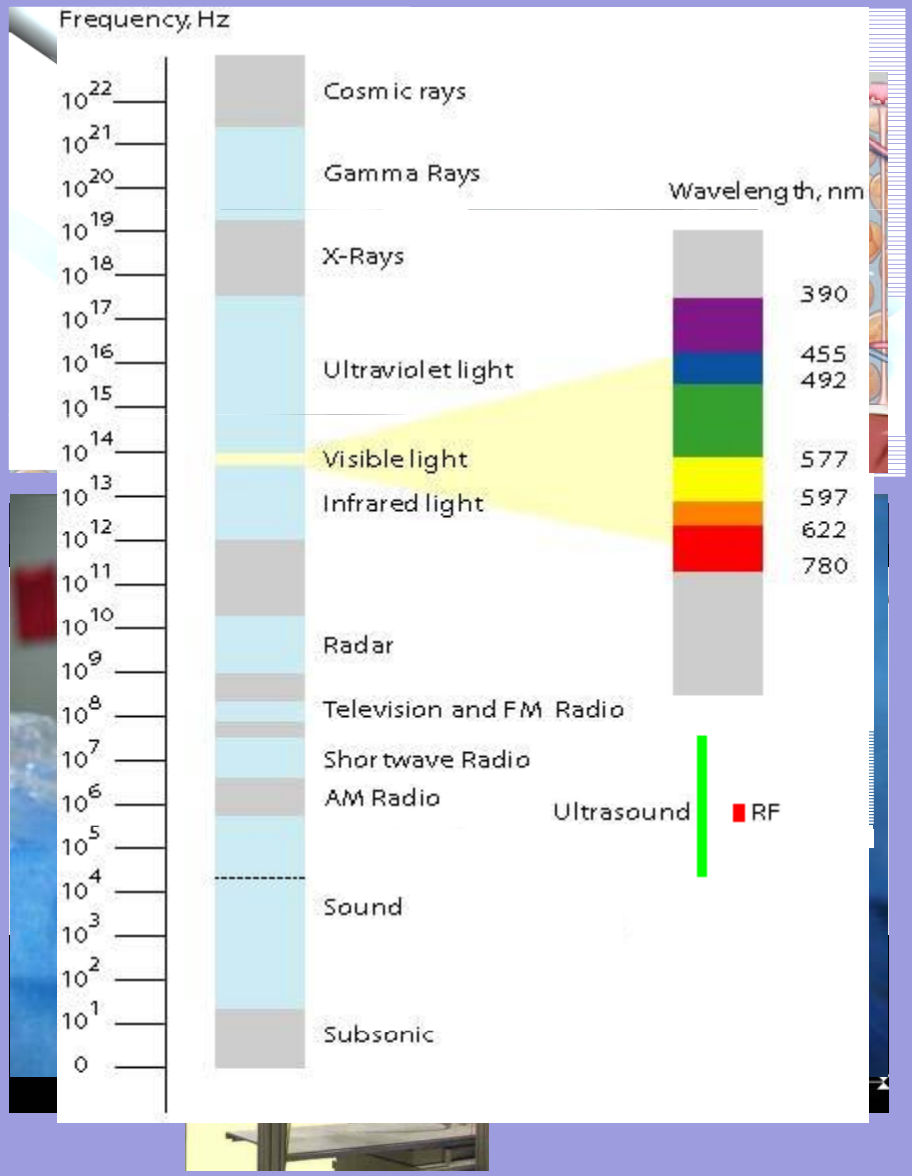
Outline

- Introductory Materials
- Background
  - Ultrasound mechanisms in medicine
  - Application to adipose tissue
- Two-Part Experimental Approach
  - Low Frequency (36kHz) invasive treatment
    - Viability of aspirated adipocytes and stem cells
  - High Frequency (1MHz) non-invasive treatment
    - Cellular effects and implications
- Conclusions

Introduction, or Why I love Ultrasound

- Ultrasound is an incredibly diverse technology
- Application of ultrasound in medicine has revolutionized both diagnosis and treatment of disease (“Billions and billions…”)
- By tailoring the temporal and spatial characteristics of the ultrasound energy, radically different outcomes are possible
- This work presents two types of ultrasound treatments for adipose tissue

Background: Ultrasound Spectrum



Medical Ultrasound:
20kHz to > 20MHz

Therapeutic/Surgical ultrasound:
20kHz to 2MHz

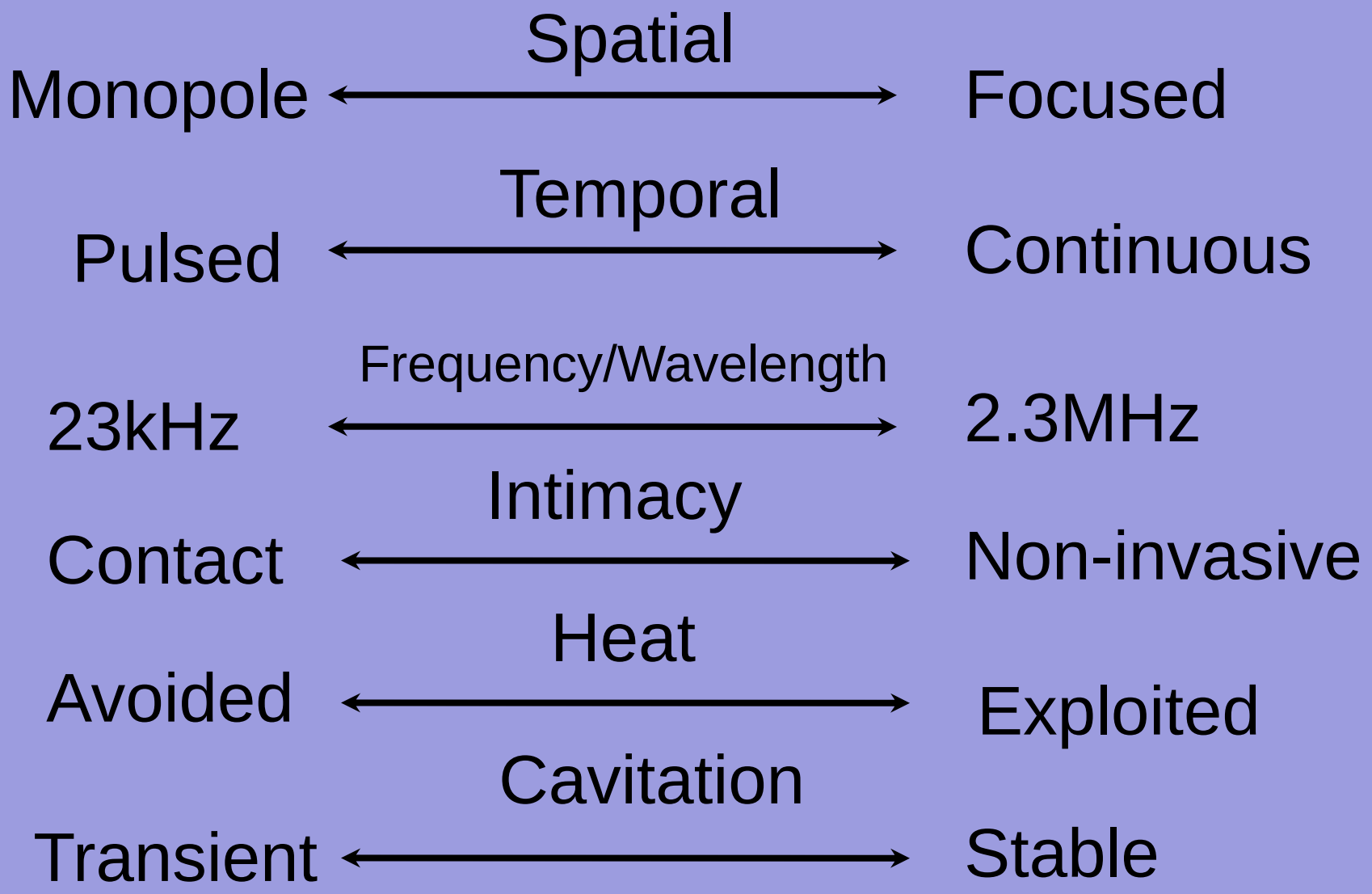
Diagnostic Ultrasound:
2MHz to >20MHz

“Low” frequency Ultrasound:
< 250kHz

Wavelengths:
10’s of cm to <0.1mm

Can be focused or unfocused

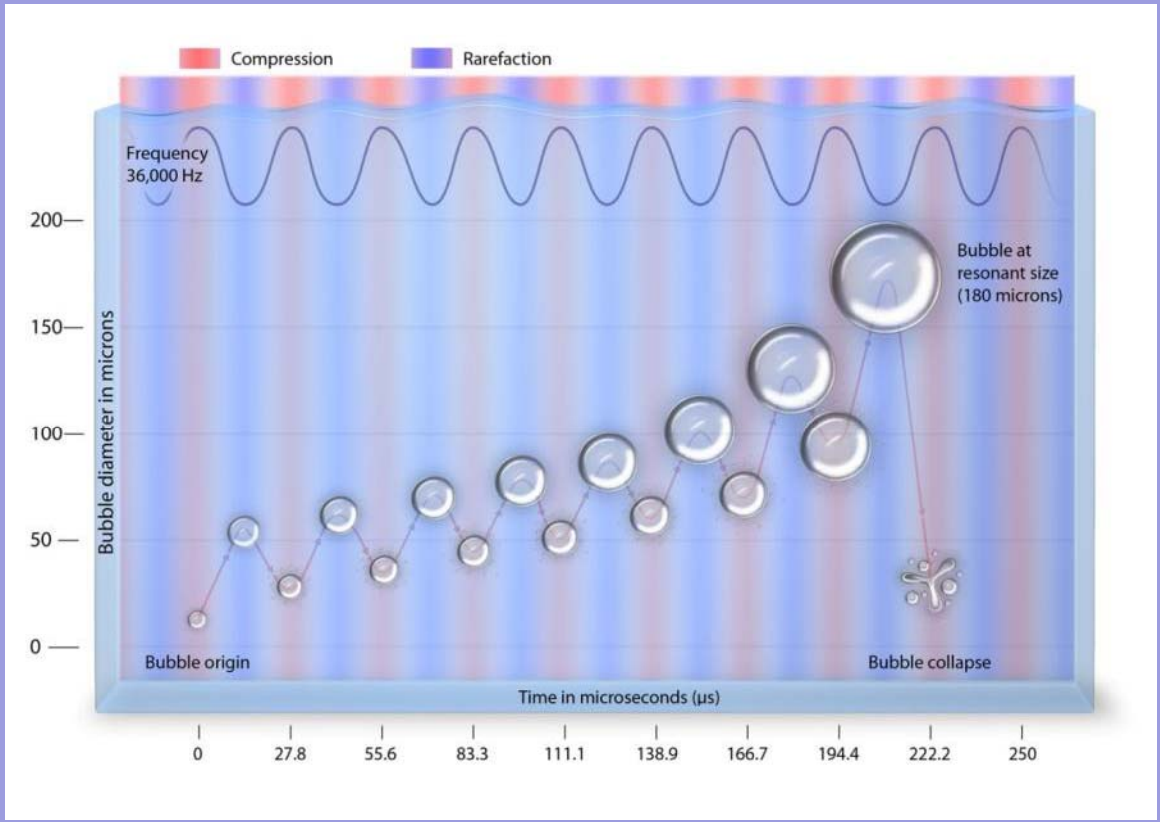
Ultrasound's Diversity: Multiple Approaches



Ultrasonic Interaction with Tissue

- Thermal
  - Absorption or Frictional/Viscous Heating
- Cavitation
  - Action of microbubbles, Transient or Stable
- Radiation Force
  - Net force on tissue due to passage of waves
- Acoustic (Micro)Streaming
  - Induced viscous flow around cells
- Isolate or blend effects based on design
  - Frequency, focusing, intensity, temporal
- Biological / Chemical effects
  - Vasodilation, sonopheresis, improved lymph flow, muscle relaxation, reduced inflammation, and pain relief. Short-term change in pH and cell membrane permeability

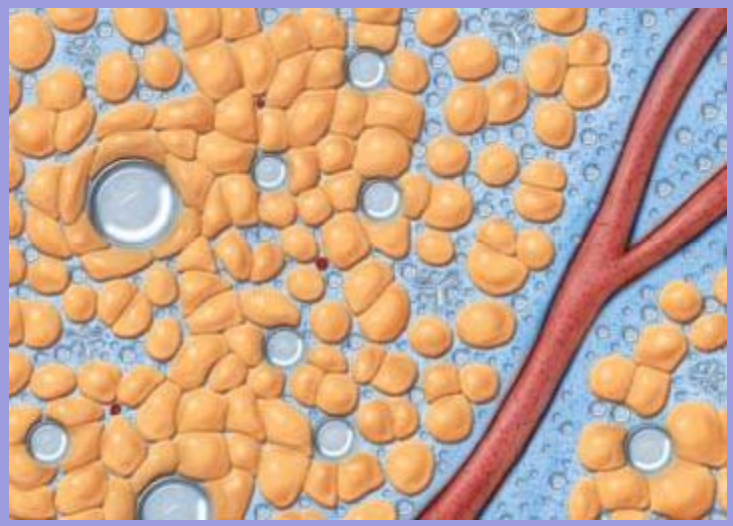
Low Frequency Ultrasound: Bubbles



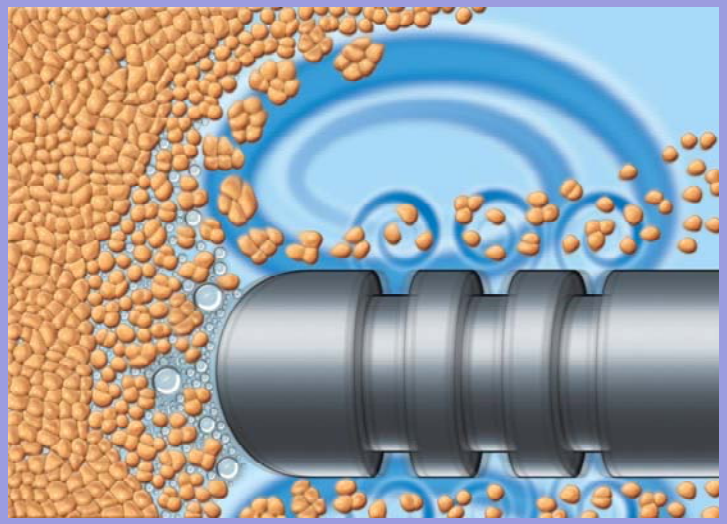
- **CAVITATION**: the creation and action of air or gas bubbles in a liquid
- Ultrasound causes cyclic compression and rarefaction (squeezing and pulling) on very small bubbles (cavitation nuclei)
- Bubbles grow until they reach “resonant” size, at which point they collapse and the process repeats.

Low Frequency Ultrasound and Adipose Tissue

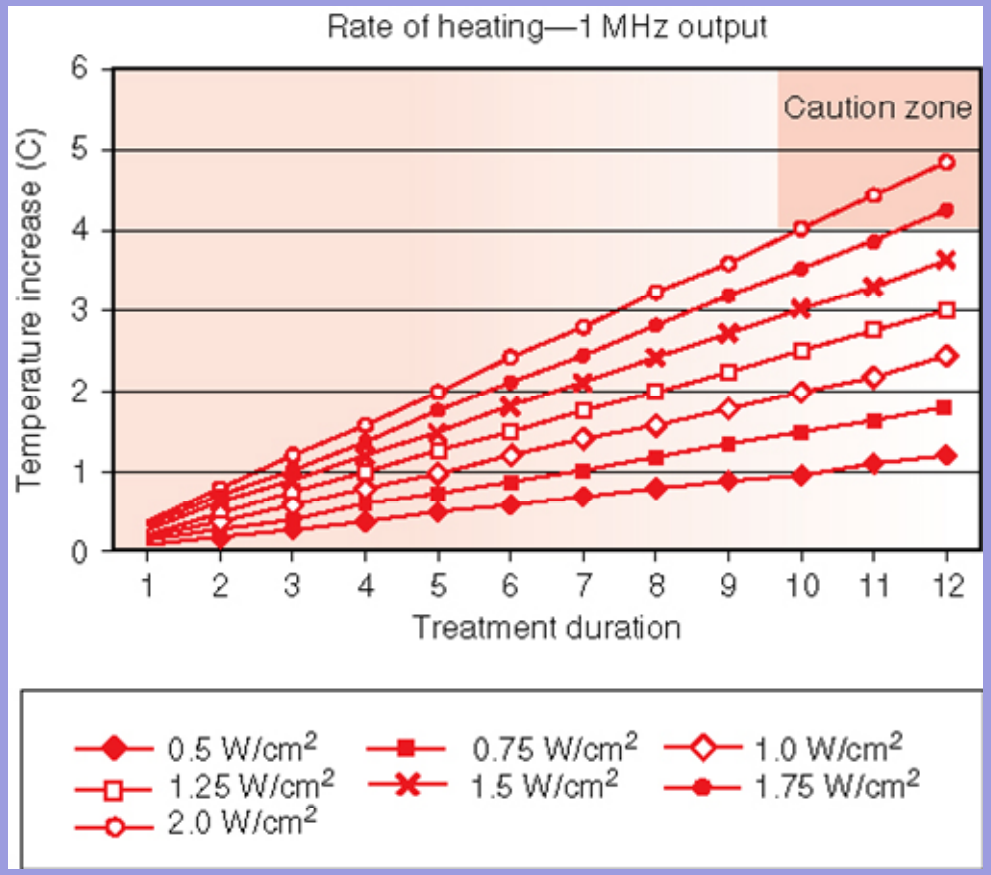
- **Cavitation** nuclei interspersed among the fat cells grow and expand, forcing the fat cells apart.
- After reaching their resonant size, the bubbles collapse, pulling the fat cells from their matrix.



- Once the fat is loosened, **Acoustic Streaming** causes it to mix with the tumescent fluid to form a suspension.
- Near the vibrating tip, these forces cause intense localized swirling to further break up the fat into small clusters of cells.



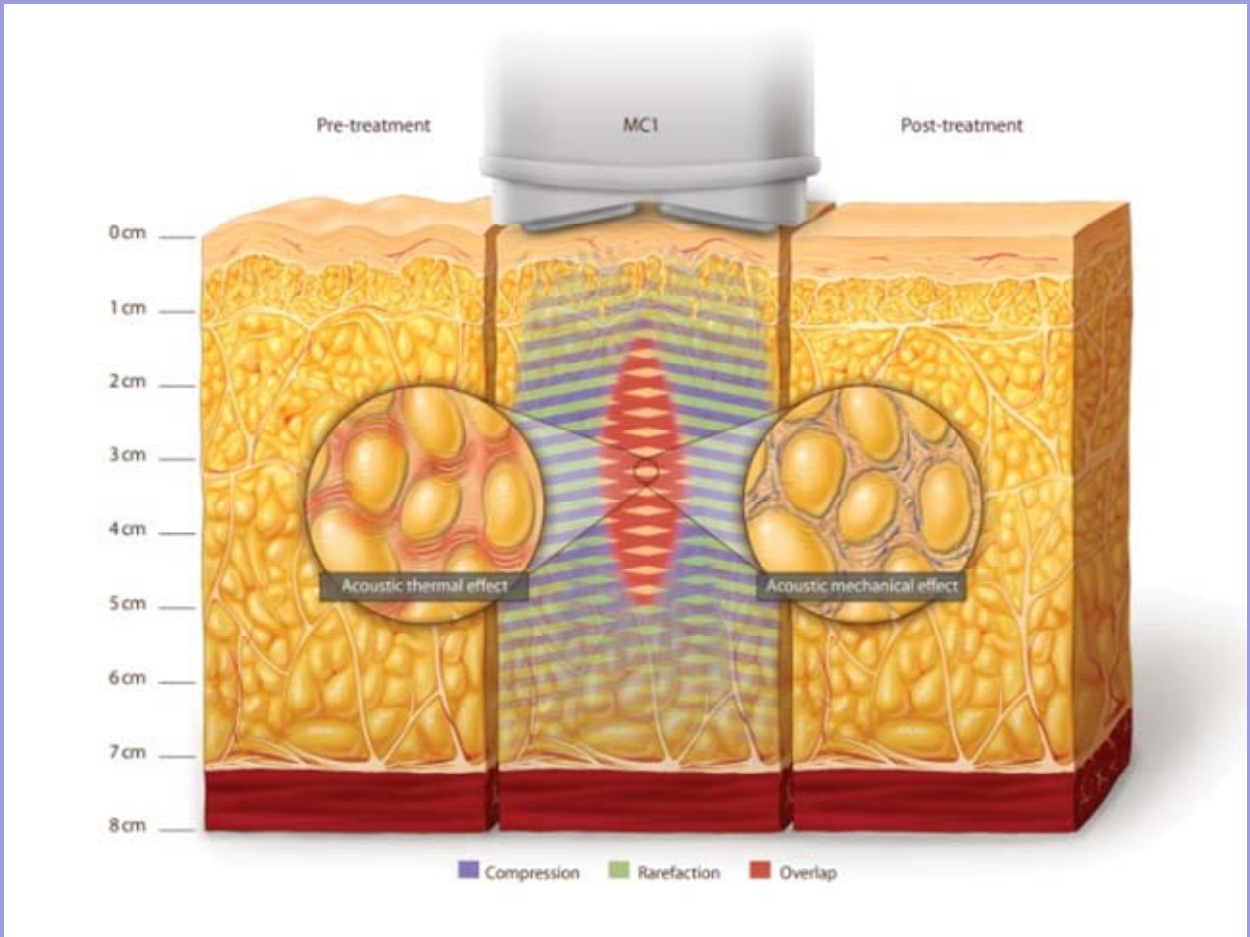
High Frequency Ultrasound: Thermal Effects



- Temperatures can rise a biologically significant amount in a few minutes
- 1MHz ultrasound, and the heat that it creates, will effectively permeate about 4-5cm into tissue
- At these power levels, other effects such as radiation force also occur

High Frequency Ultrasound and Adipose Tissue

- Unfocused ultrasound (1-3MHz) is well established as a treatment modality through induced heating and mechanical micro-stimulation



Two Part Experimental Approach

Low Frequency

- Use the 36KHz VASER® system for lipoaspiration
- Harvest samples from five patients
- Examine the viability of the harvested adipocytes for reimplantation[1]
- Process the aspirate to get stem cells[2,3,4] and compare to other approaches

High Frequency

- Use the 1MHz, dual transducer VASERshape for external treatments
- Treat three Yorkshire pigs
- Attempt to track what happens to lipids
- Determine effects on cellular structures

[1]*Aesthetic Surg J* 27:641–655, 2007

[2]*Keio J Med.* 2005;54(3):132-141

[3]*Mol Biol Cell.* 2002;13:4279–4295

[4]*Plast. Reconstr. Surg.* 124: 65, 2009

System Comparison

Low Frequency (VASER)

High Frequency (VASERshape)

LF Ultrasound: Materials and Methods

- Equipment settings:
  - VASER system setting: 60-70% amplitude in the pulsed (VASER) mode
  - VentX aspiration system setting: 15 inHg or less vacuum
  - 3mm cannulae
- Consented patients undergoing elective liposuction procedures.
- Patient selection criteria:

Male or female; Age 20-50
Good health; ASA Class I; BMI < 30
Non-smoker preferable
Preferred areas: abdomen, flanks, inner and outer thighs

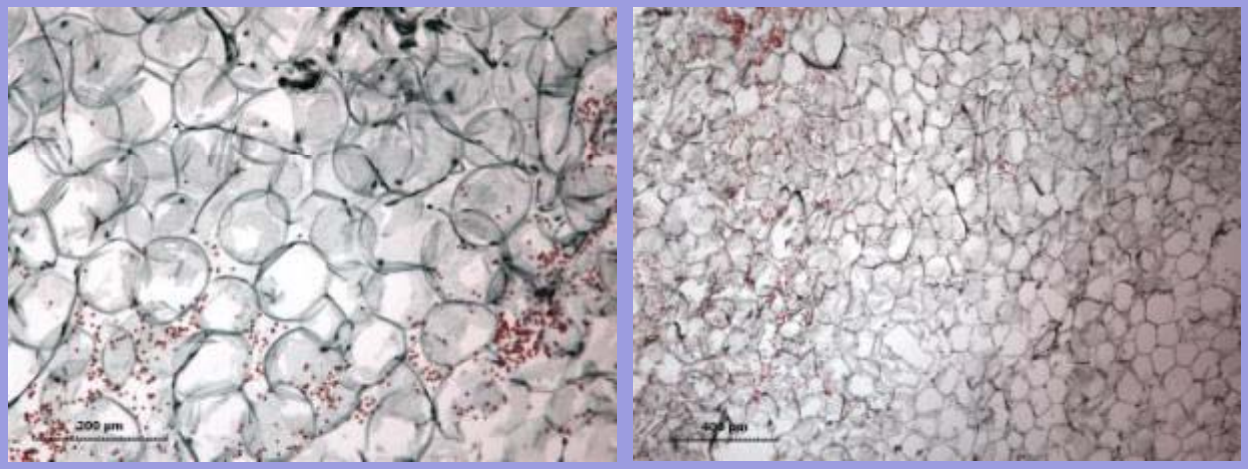
Exclude: back, chest, arms, calf, superficial sculpting
No revisional surgeries

Run ID #	Gender	Age	Harvest Site	Volume	Experiment Date	Physician
V-2524-1	F	24	Abd, flanks	1.7 L	12/14/2010	A
V-2554-2	F	28	right flank, left flank	730 mL	1/21/2011	B
V-2588-3	F	45	flanks, thighs	800 mL	2/25/2011	C
V-2602-5	F	43	flanks/inner thighs	800 mL	3/17/2011	A
V-2646-7	F	38	thighs	1.5 L	419/2011	D

Note: samples were collected from two other patients who were determined not to have met the study selection criteria (age and sex)

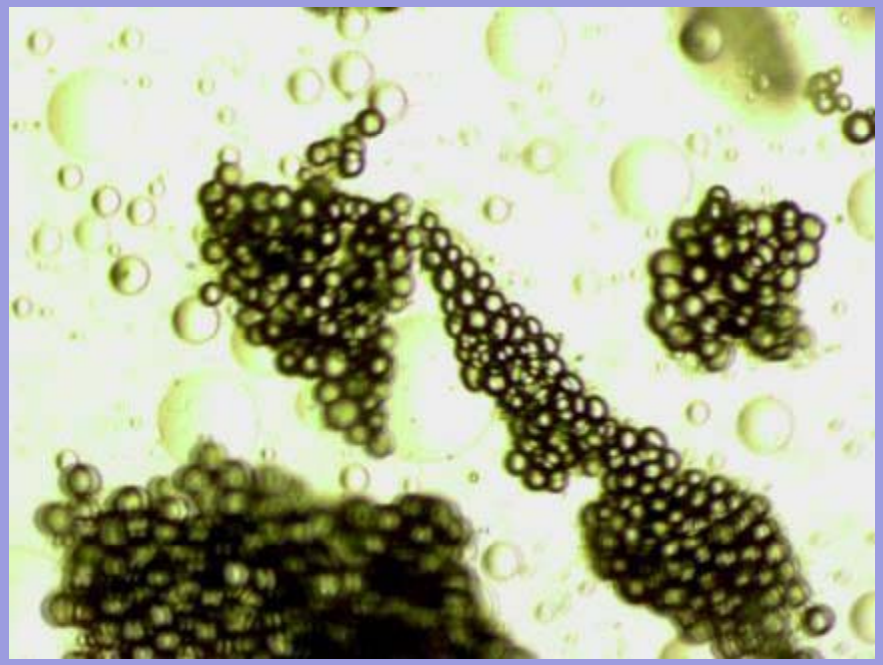
Typical Aspirate & Gross Morphology (H&E)

"Clean" aspirate with little free lipid



Tissue comparable to syringe or SAL derived samples, showing mature unilocular adipocytes with intact membranes

Intact Cell Images



Intact fat cell clusters (Thermogenesis)

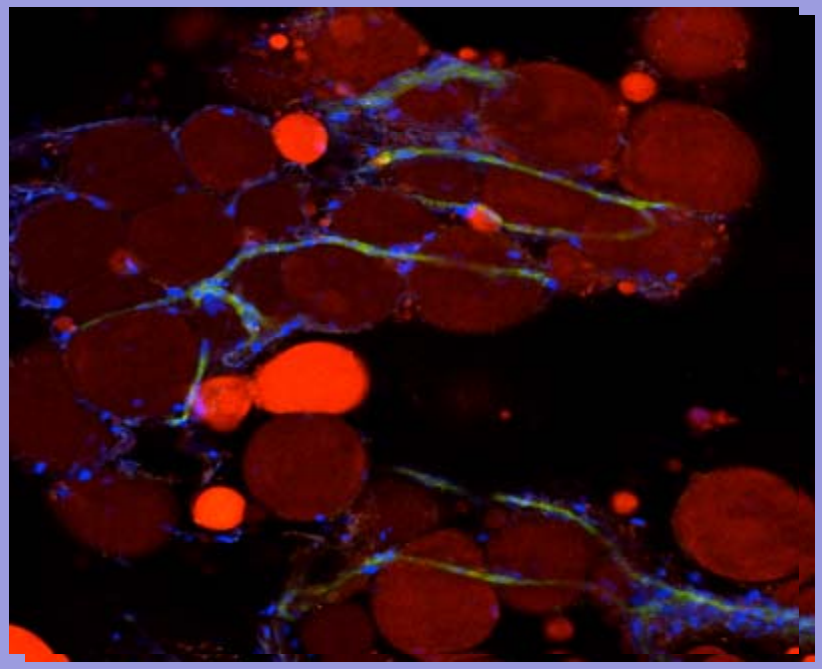
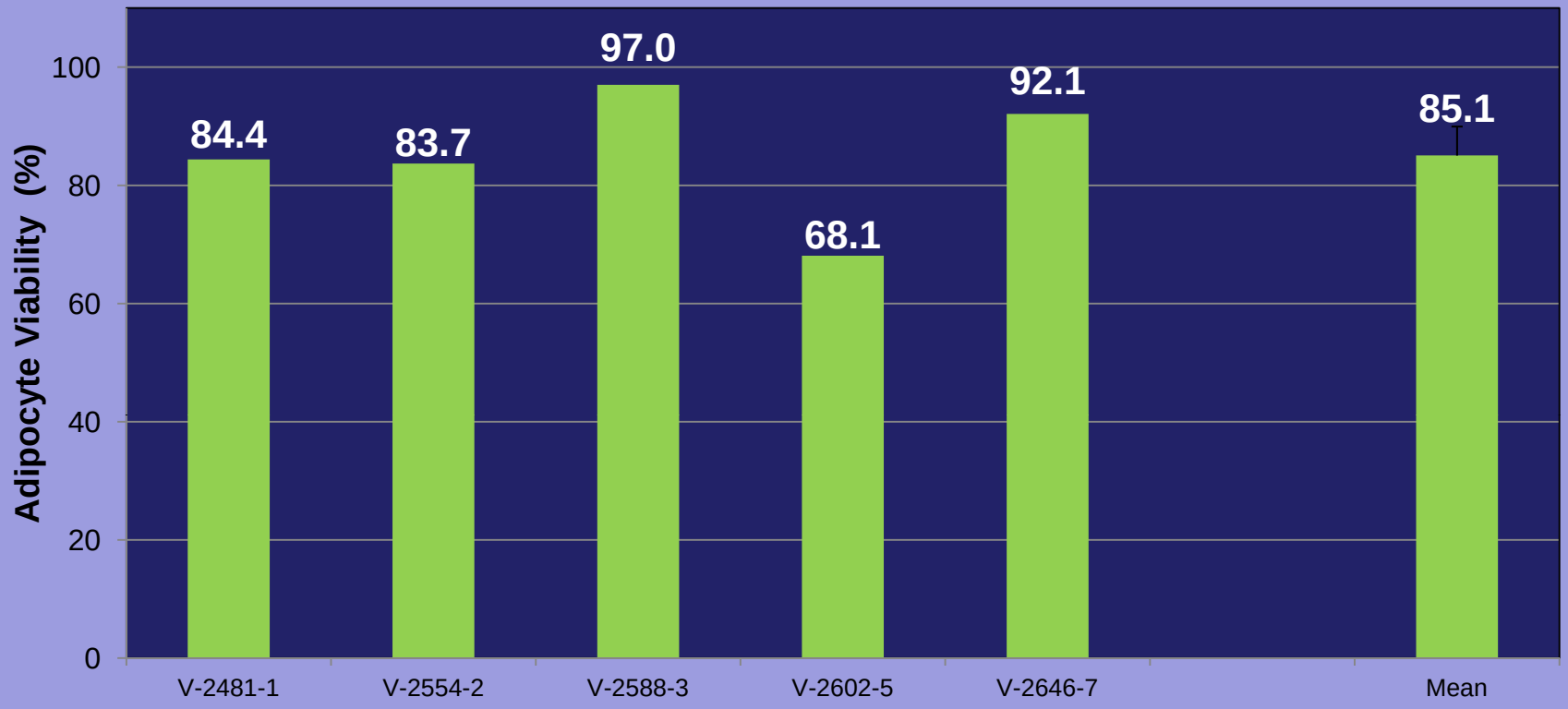


Image shows intact fat cells (dark red), free lipid (bright red), ADRCs (blue) and collagen (green) (UPMC)

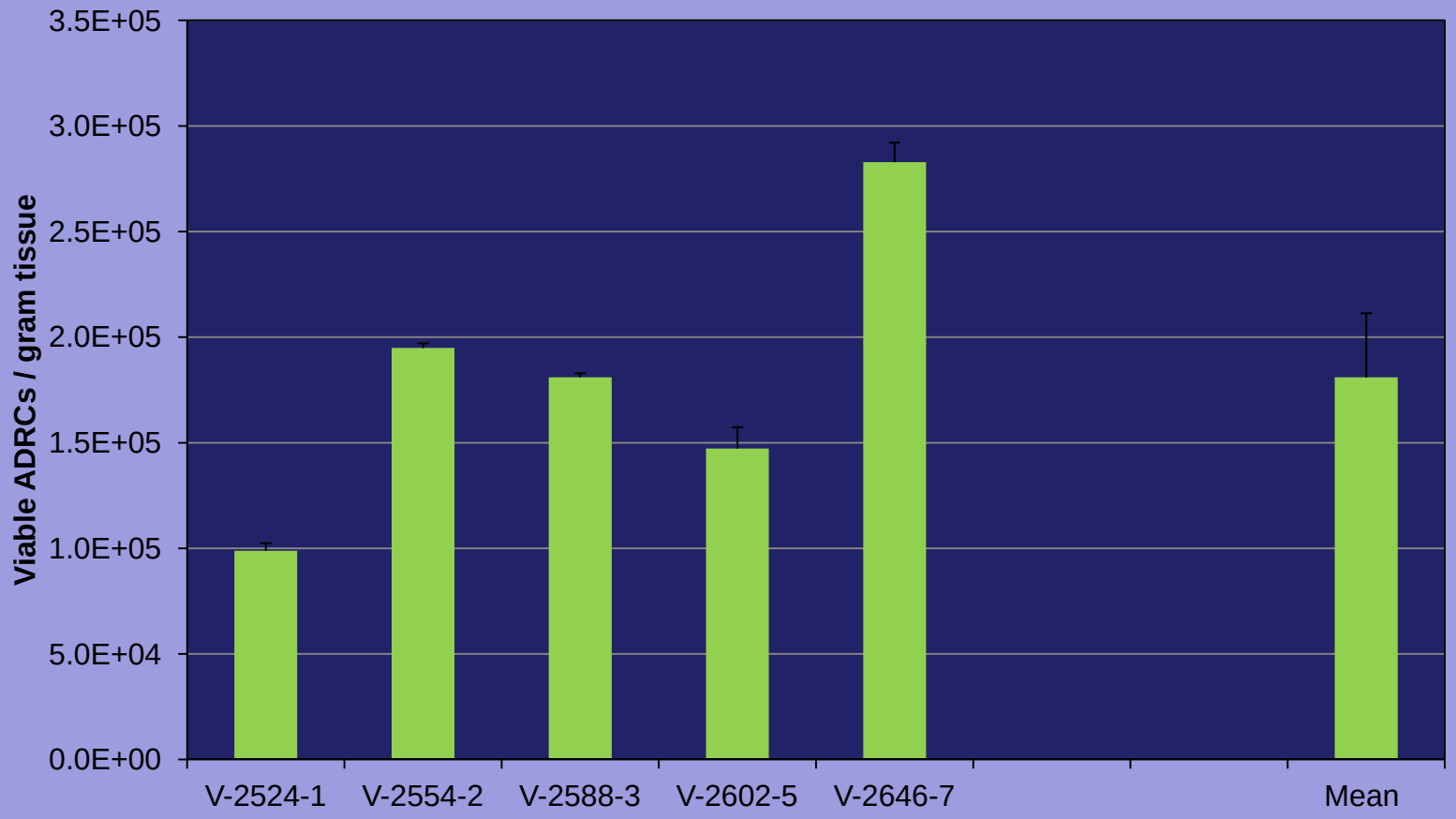
Adipocyte Viability: Functional Assessment by Lipolysis Assay



Lipolysis assay determined adipocyte viability by measurement of metabolic activity known to directly correlate to viability. An agonist of glycerol release (isoproterenol) was used, and free glycerol content was determined by spectrophometry

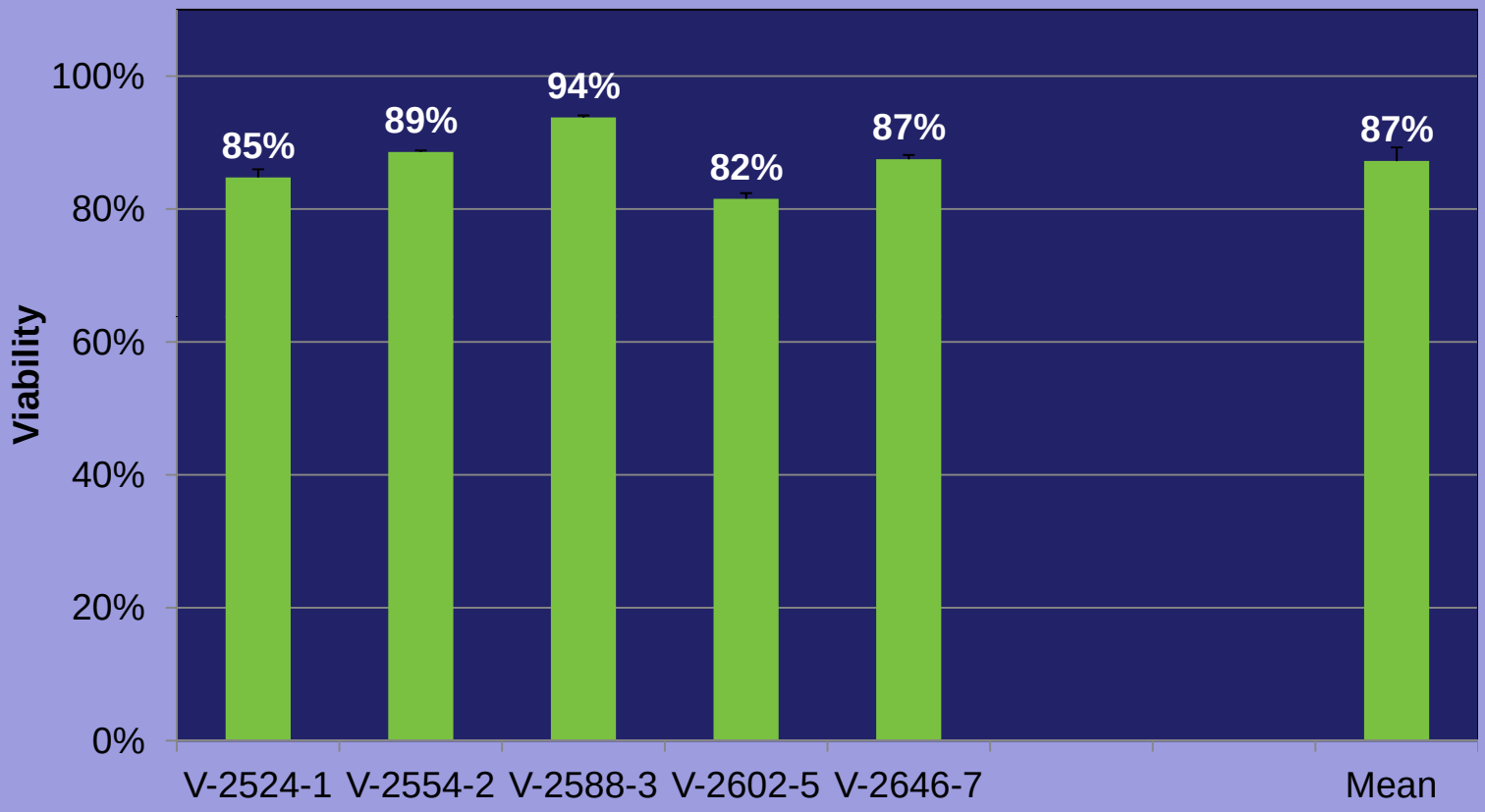
Mean value of **85.1%** (comparable to published SAL results), independently confirmed by UPMC, Thermogenesis

Viable ADRC density



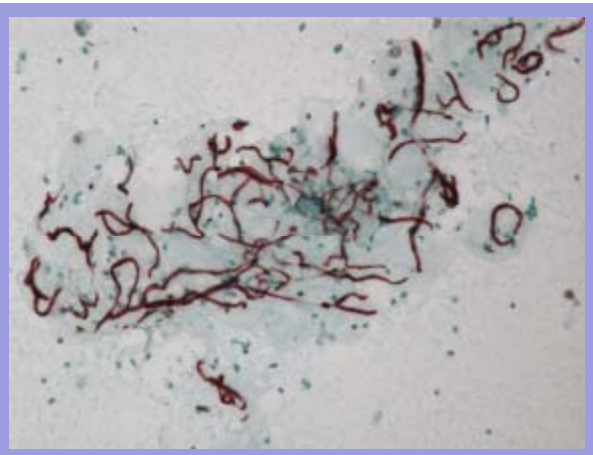
Adipose tissue was processed using the Celution® 800/CRS system. Resuspended ADRCs were counted using the NucleoCounter™ system to determine the number (**$1.81 \times 10^5 \pm 0.68 \times 10^5$ cells/gm**) and relative viability of the cells (next slide).

Viability of ADRCs

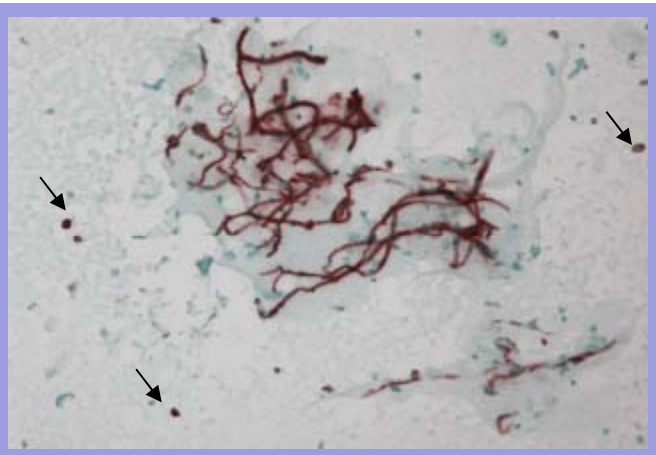


Again, values were independently confirmed by other research groups.

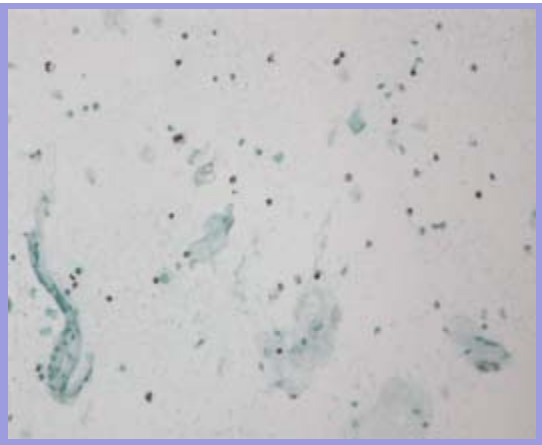
Immunostaining of loosely adherent cells/components



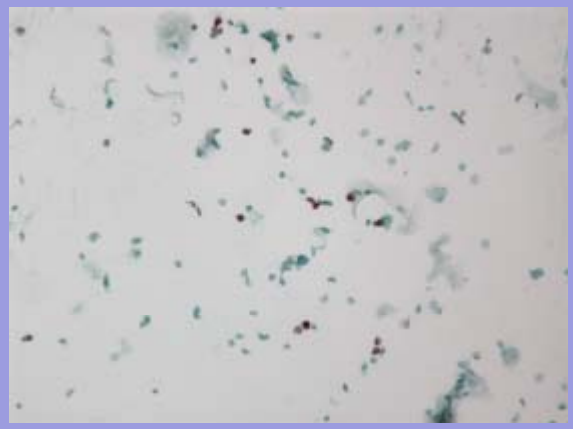
CD 31



CD 34



CD45 positive (hematopoietic)



CD68 positive monocyte/macrophages

Arrows denote individual CD34 positive progenitor cells.

CFU-F percentage of 0.33% fell within historical range for syringe techniques.

LF Experiment: Discussion

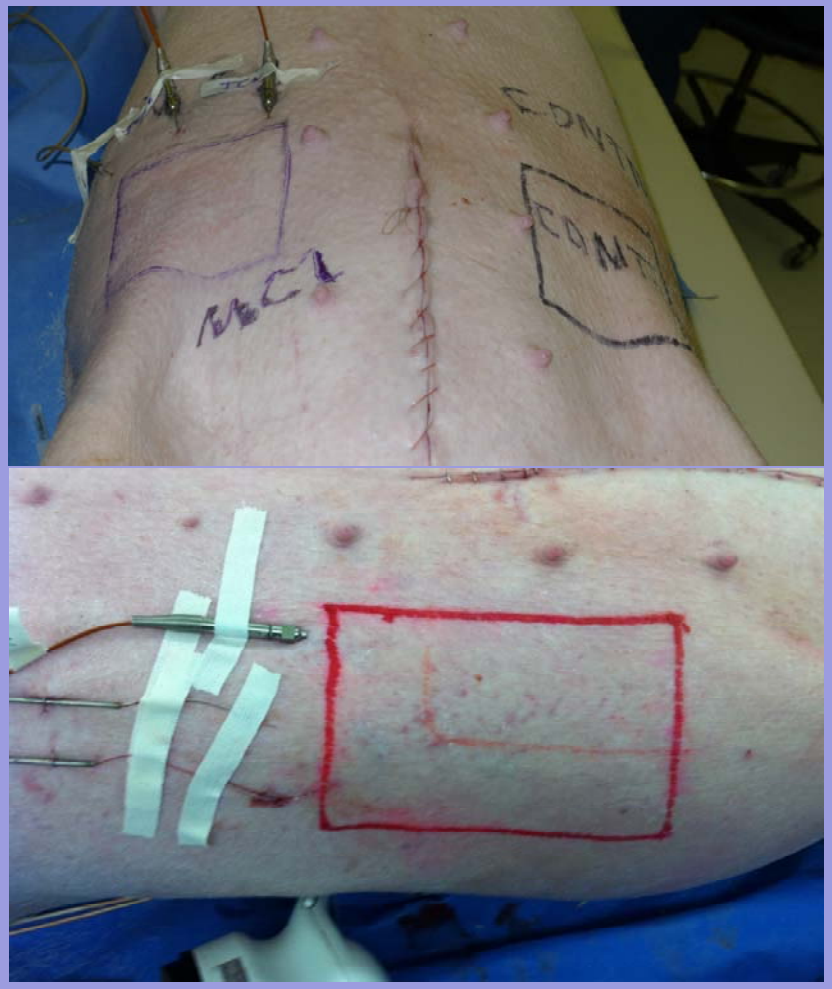
- The laboratory data indicate:
  - density and viability of both the adipose cells and the ADRC component of the lipoaspirate were comparable to standard (non-ultrasound) methods.
- This would support a proposed mechanism of action that does not destroy cell membranes, but selectively dislodges adipocytes from the tissue matrix (stable cavitation, acoustic streaming)
- Specifically the system uses:
  - higher frequencies (36.6kHz vs 24kHz)
  - lower amplitudes (73μm vs 120μm)
  - custom drive conditions (VASER mode)
  - grooved solid probes[1]

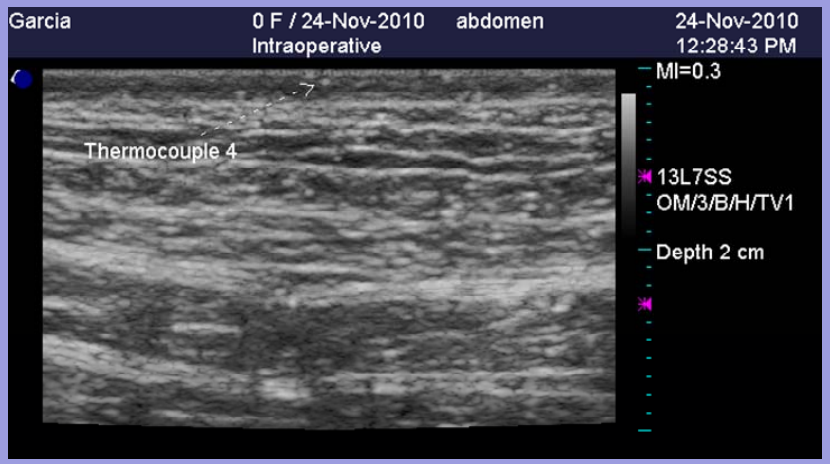
[1]US Patent 6,368,299

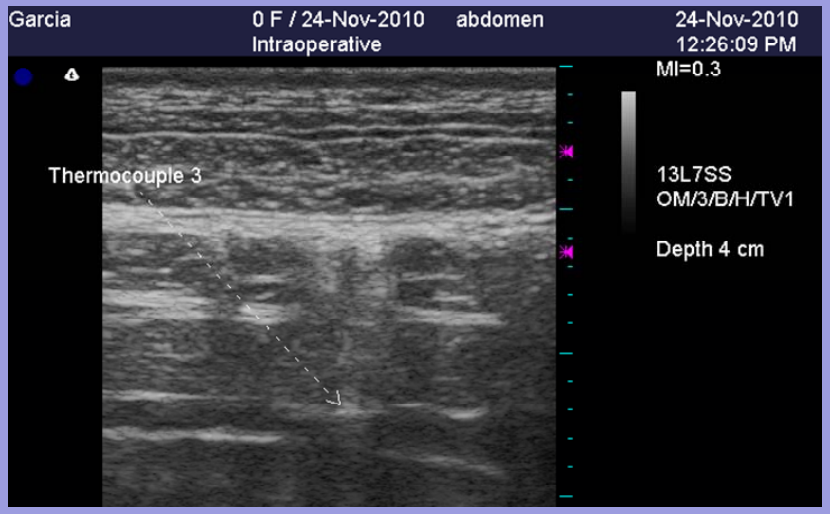
HF Ultrasound: Materials and Methods

- Equipment settings:
  - VASERshape system setting: mulitiple treatments at typical clinical setting 5W/cm^2
- Three anesthetized Yorkshire pigs (50+ kg)
  - Full IACUC approval for acute study
  - One side treated, other side control
  - Implanted thermocouples (2, 14, and 32mm)
  - External IR camera
  - Harvest skin, fat, muscle on both treated and control sides
  - Harvest lymph fluid pre/post; biopsy of lymph tissue treated/control
- Analysis of tissue
  - Histology (H+E, TriChrome, NADH, Oil Red O)
  - Triglyceride values for blood and lymph fluid samples

HF Ultrasound: Materials and Methods

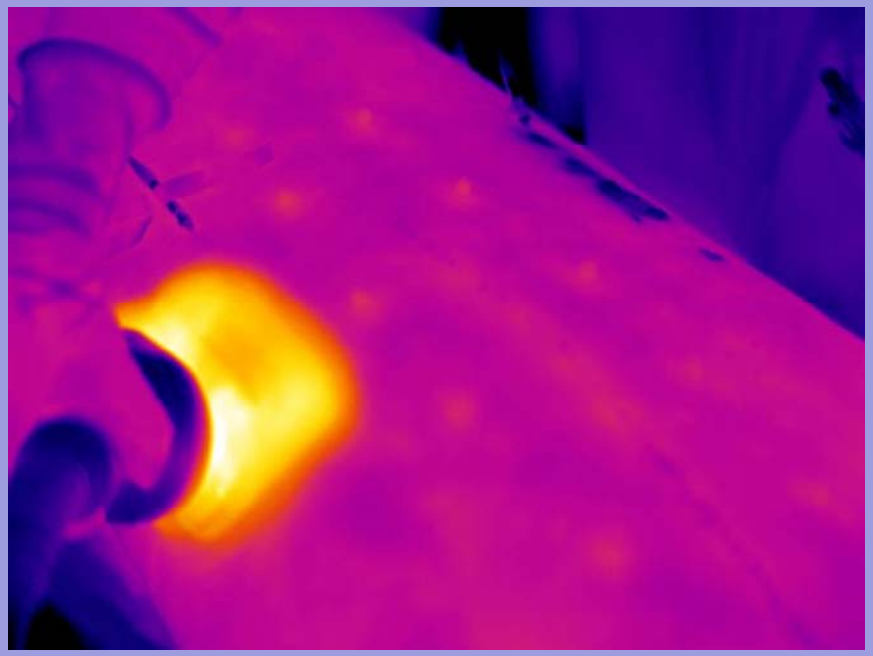






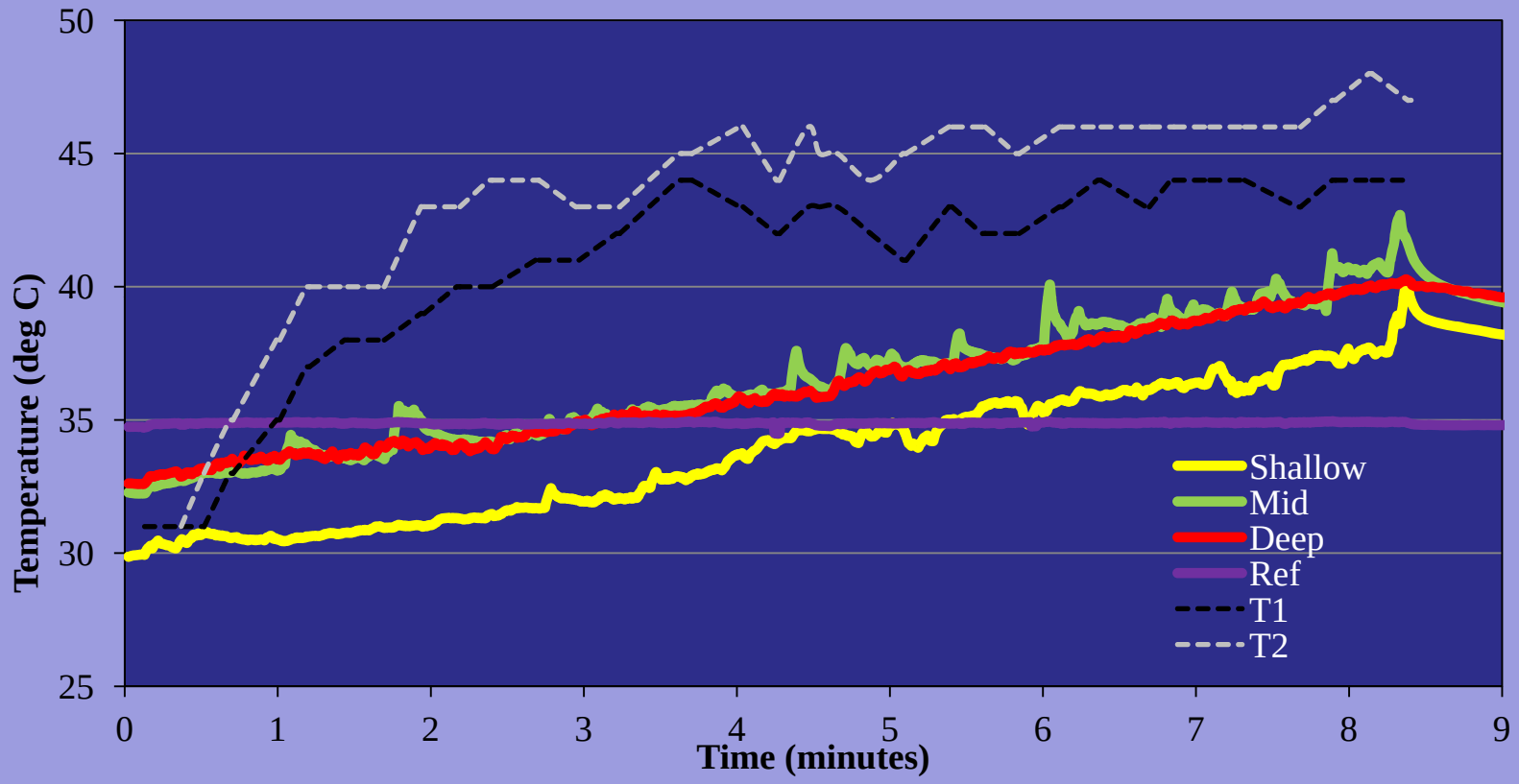
Ultrasonic guidance of thermocouple using TouchView system

Infrared Video Imaging



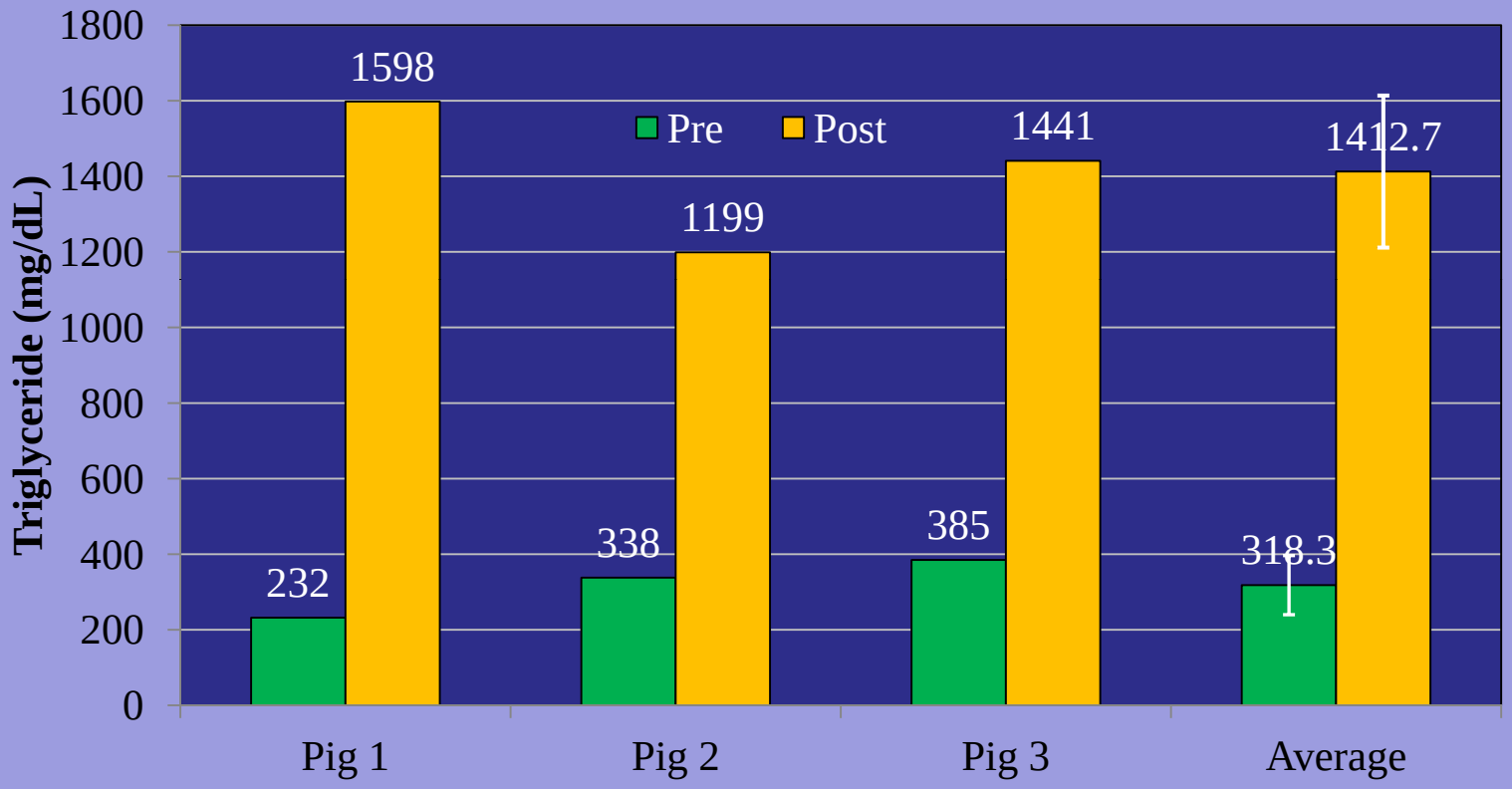
- Warmer areas show up in orange; cooler areas in purple
- Image shows pig belly, treated area, operator arm holding handpiece on left side
- Operator treated the skin surface to produce uniform heating over a large surface area
- Temperature reached level necessary to cause skin retraction

Temperature Results



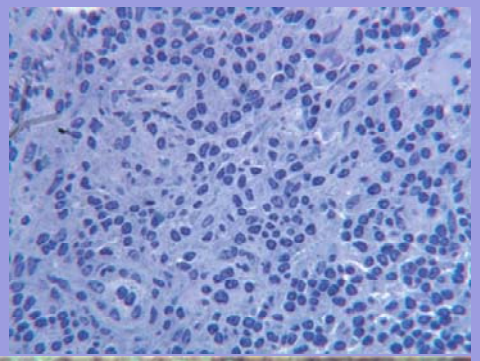
Uniform heating down to at least 30mm

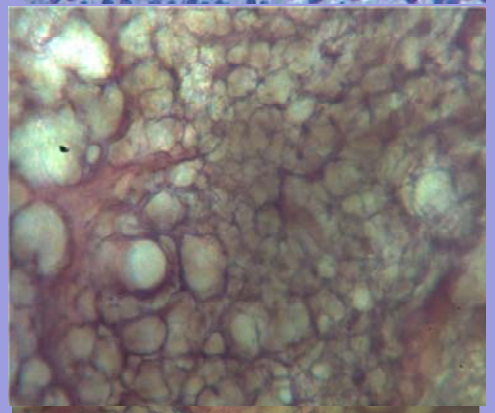
Change in lymph Triglycerides

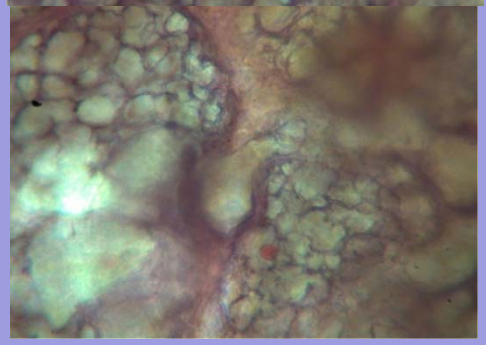


Blood triglyceride levels also showed a slight increase

Samples from lymph nodes





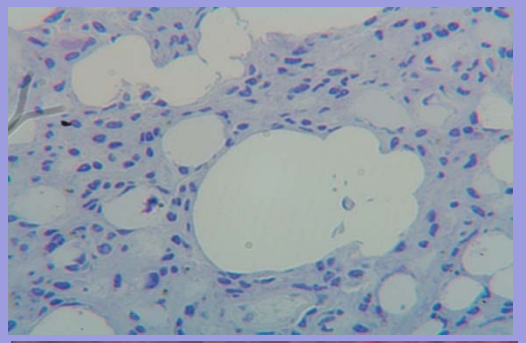


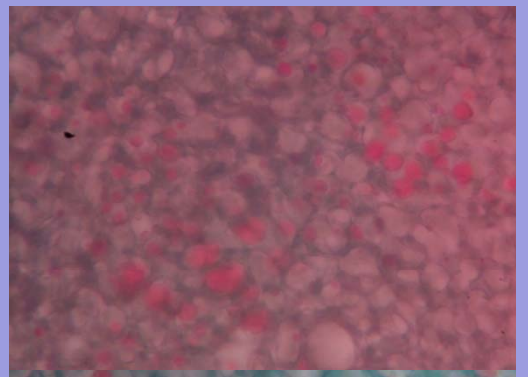
Left - Control

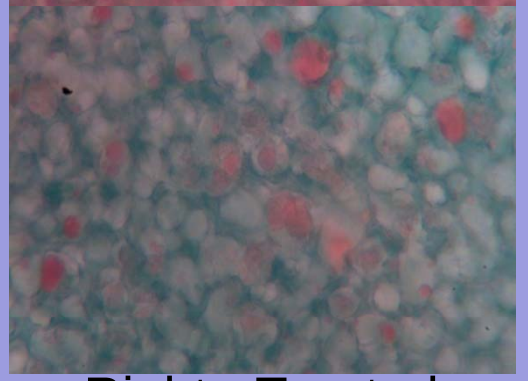
Images and staining show that lipids have migrated from the adipose compartment into the lymph system

EM of fat cells shows minor disruption of cell walls

All other histology images indicate no changes to muscle or skin







Right - Treated

HF Experiment: Discussion

- Animal studies demonstrate:
  - Subcutaneous and deeper heating effects from the ultrasound exposure
  - No apparent harm to the adipose cells, nor to the overlying skin or underlying muscle layers
  - Adipose cells release their internal lipid contents, which are flushed into the lymphatic system
- These results support a proposed mechanism of action, which includes a (predominant) thermal effect (dramatically increasing the metabolic rate of the adipocytes), acousto/mechanical action on the cells, and acoustic streaming forces on the freed lipids

Wide range of ultrasound effects

Low Frequency

- The 36kHz VASER® system for lipoaspiration
- Harvested cells, both fat and regenerative, were plentiful and viable
- Mechanism of action: stable cavitation, acoustic streaming (non-destructive)

High Frequency

- The 1MHz, dual transducer VASERshape for external treatments
- Deep, uniform tissue heating, with no lysis of fat cells or damage to surrounding tissue
- Lipids released and forced to the lymphatics
- Mechanism of action: thermal and micromechanical

Acknowledgements

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- Thermogenesis

THANK YOU!