Ultrasound-Mediated Drug Delivery using Echogenic Liposomes

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Seminar Overview

- Definition of the clinical problem
- Targeted nanoparticle development
  - Echogenic liposomes (ELIP)
- Ultrasound-enhanced drug delivery (1 MHz)
  - Ex vivo arterial flow model
- Thrombolytic efficacy of tPA-loaded ELIP (120 kHz)
  - Time-lapse thrombolysis videos in vitro
- Thrombolytic efficacy of tPA and Definity® contrast agent (120 kHz)
  - Ex vivo arterial flow model
- Sustaining stable cavitation with contrast agents

Cardiovascular Disease

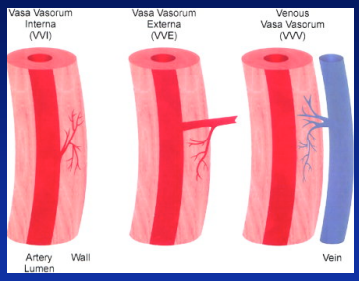
- #1 Cause of Death and Disability Worldwide (WHO)
- Accounts for 34.3% of all deaths in the US (Lloyd-Jones D, et al. Circulation, 2009)
- Mortality and morbidity declining, yet remain high due to poor understanding of atheroma development (Heidenreich and McClellan, Am J Med (2001).

Development of US techniques

- Evaluation of the progression of atherosclerosis
- Identification of the morphology of atheroma and thrombus

Landini et al., UMB 1986
Barzilai et al., Circ Res 1987
Picano et al., Circulation 1988
Jones et al., Ultrasonic Imaging 1987 & 1989
Ng et al., Circulation 1993

Functional anatomy of vasa vasorum



Gossl M, et al. Am J Physiol Heart Circ Physiol, (2003)

Development of Ultrasound Contrast Agents Targeted to Atheroma or Thrombus

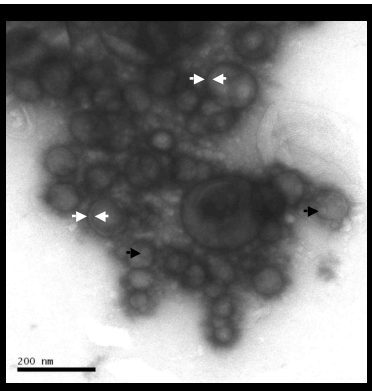
- Diagnosis - Identification of plaque vulnerable to rupture
- Therapeutics - thrombolytic delivery
  - Ischemic Stroke
  - MI
  - DVT
  - AV Fistula Maintenance for Dialysis Access

Molecular "Velcro" Targeting Strategies

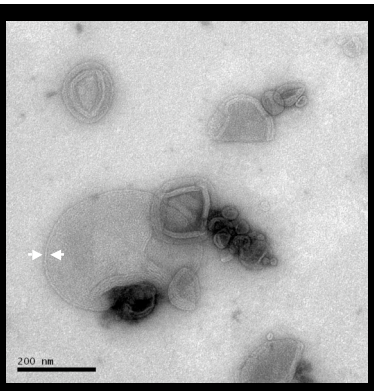
- Linear hexapeptide coupled to lipid (Aerosomes, ImaRx, Tucson, AZ, USA)
- Antibodies targeting Glycoprotein IIb-IIIa receptor (Bracco Research SA, Geneva, Switzerland)
- Avidin-Biotin linkage to target fibrin (Lanza *et al. Circulation*, 1996) or arterial tissue factors (Lanza et al. *J. Am. Soc Echo*. 2000).
- ICAM-1 to target atheroma (Mastrobattista *et al. Biochim Biophys Acta*, 1999)
- Inactivated rt-PA in ELIP to target fibrin (Klegerman *et al., J Liposome Res*, 2008)

Targeted Immunoliposomes

Klegerman ME *et al.*
Biochim. Biophys Acta - Biomembranes (2007)
J. Liposome Res., (2008)
J. Controlled Rel. (2010)

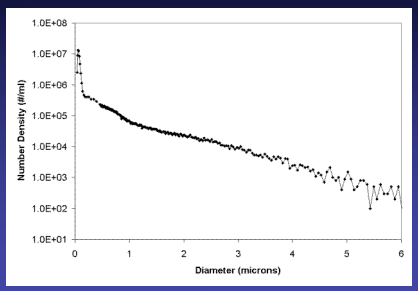


JEOL 1200 TEM at 60 kV captured with Gatan BioScan 792 CCD camera.

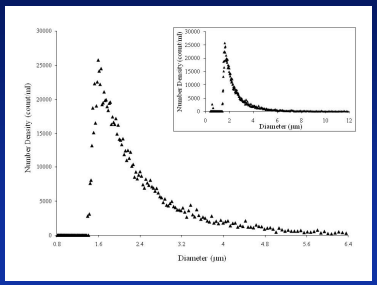


JEOL 1200 TEM at 60 kV captured with Gatan BioScan 792 CCD camera.

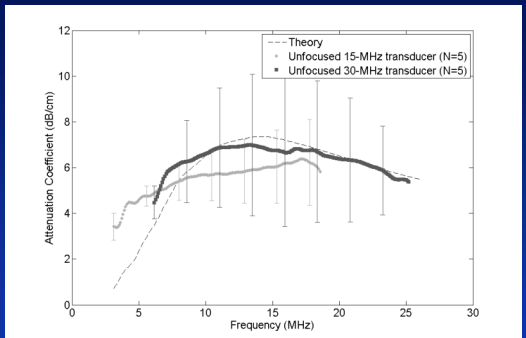
ELIP Size Distribution

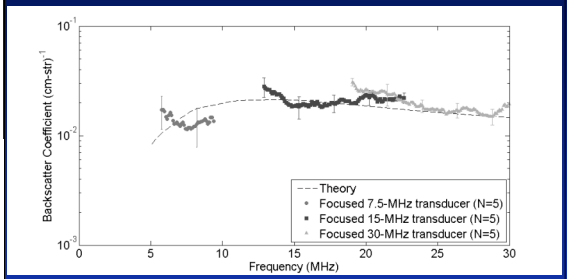


Size Distribution of rt-PA-ELIP

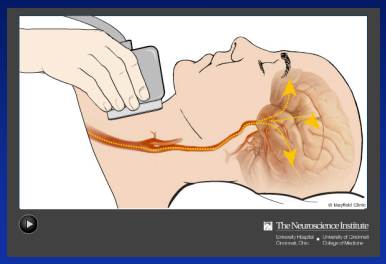


Smith, DA *et al. Ultrasound Med. Biol.*, (2010)

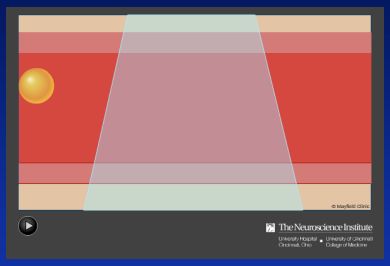




Ultrasound Controlled Drug Delivery Paradigm:

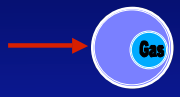


Ultrasound Controlled Drug Delivery Paradigm:

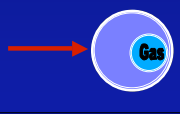


Interaction of UCA with US

- Acoustically Driven Diffusion – forced diffusion of gas into the medium



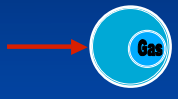
- Rapid Fragmentation – shell fragments thereby liberating gas



Smith DAB et al., UMB, 2007

Interaction of UCA with US

- Primary Radiation Force - net displacement of UCA in the direction of wave propagation

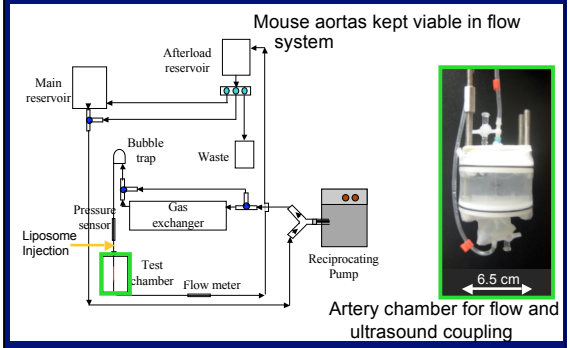


Drug Delivery with 1-MHz CW US Using ICAM-1-targeted ELIP

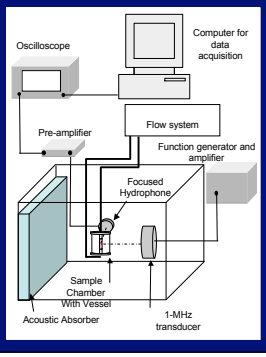
- Artery permeability after ultrasound + bubbles
  - Red blood cells and dyes observed leaking[2]
  - Could work for therapeutics as well
- Hypothesize a stable cavitation mechanism[3]
- Objective: To investigate potential for ultrasound-induced stable cavitation to enhance delivery to the artery wall[4]

Miller et al., *Journal of the American College of Cardiology*, 2006.
Datta et al., *Ultrasound in Medicine and Biology*, 2006.
Hitchcock et al., *J. Controlled Release*, In press 2010.

Materials and Methods: Flow System



Materials and Methods: Passive Cavitation Detection



Materials and Methods

- Protocol approved by IACUC of U. of Cincinnati
- Removed aortae of 16 mice at 17 – 24 weeks
- Flow of 0.5% bovine serum albumin, 3.4 mL/min
- 30-second bolus of ELIP at 1.8 mg lipid/mL
  - Fluorescently labeled with Rhodamine dye
  - Targeted with antibody to Intercellular Adhesion Molecule-1 (ICAM-1)
- Allowed albumin flow to continue for 3 minutes
- Collected perivascular fluid to monitor for ELIP

Materials and Methods

- Half of arteries insonated at 1 MHz, continuous wave, 0.49 MPa_{p-p}
  - Stable cavitation regime

Numbers of arteries exposed/ Number of arteries excluded due to loss of endothelium during handling

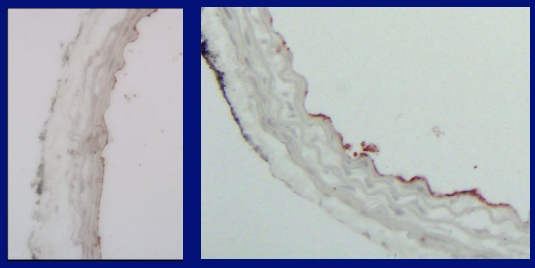
	1 MHz Ultrasound	No Ultrasound
ELIP	8 / -3	8 / -3
No ELIP (negative control)	3 / -1	4 / -2

- Additional 6 positive controls with ELIP injected into perivascular fluid

Results: Stable Cavitation

- Determined cavitation thresholds using PCD in 0.5% bovine serum albumin with:
  - ELIP
  - Flow
  - 1 MHz continuous wave
- Stable cavitation: above 0.43 ± 0.02 MPa_{p-p}
- Inertial cavitation: above 0.51 ± 0.01 MPa_{p-p}
- Confirmed stable cavitation throughout ultrasound exposure of arteries

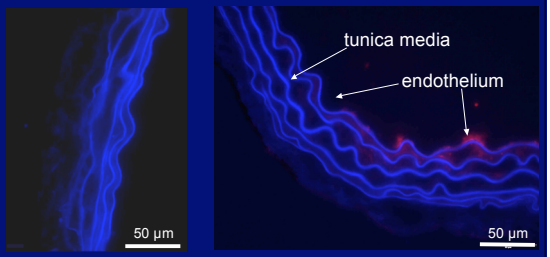
Results: Factor VIII Stain



No ultrasound

With ultrasound at 1 MHz, 0.49 MPa_{pp}

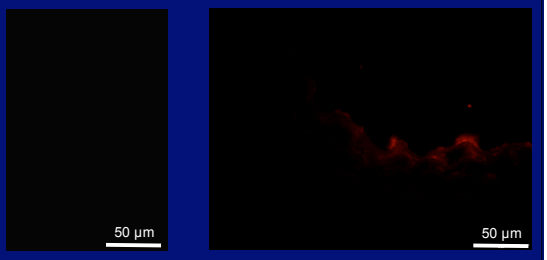
Results: Red fluorescence due to Rhodamine-labeled ELIP



No ultrasound

With ultrasound at 1 MHz, 0.49 MPa_{pp}

Results: Fluorescence Under Blue + Red Filters



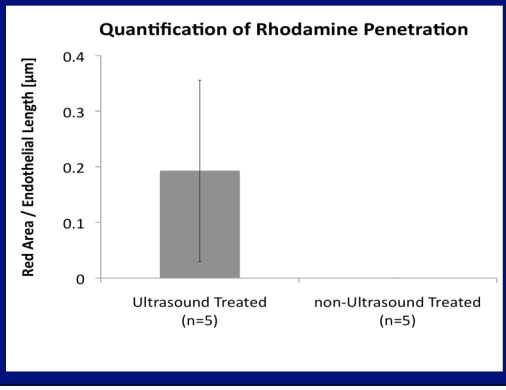
No ultrasound

With ultrasound at 1 MHz, 0.49 MPa_{pp}

Results – ELIP targeting to arteries

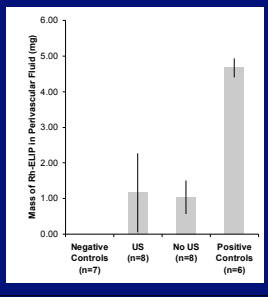
- 2 of 16 ELIP-exposed arteries excluded due to poor histological outcome
- Enhanced fluorescence on endothelium of 10 of 14 ELIP-exposed arteries
- ELIP penetration beyond endothelium in
  - 5 of 5 ultrasound-treated arteries
  - 0 of 5 non ultrasound-treated arteries
  - *p*-value of 0.008 with Fisher's Exact Test
- No full-thickness penetration

Results



Results – Analysis of perivascular fluid

- Spectrofluorometry of perivascular fluid showed high levels of Rhodamine-labeled ELIP



Discussion

- *Ex vivo* approach allows use of living tissues combined with control of variables
- Leakage from side branches limits use of small arteries for full penetration studies
- Endothelial damage to arteries observed likely due to handling
- Ultrasound-enhanced penetration into arterial wall demonstrated in this proof-of-concept study

Conclusions

- This model allows passive detection of cavitation
- Stable cavitation can be sustained in an artery with ELIP at low acoustic pressures
- Anti-ICAM-1 targets liposomes to endothelium
- 1-MHz Ultrasound at 0.49 MPa_{p-p} enhanced liposome penetration of arterial wall
- Careful selection of ultrasound variables may prevent undesirable vascular effects

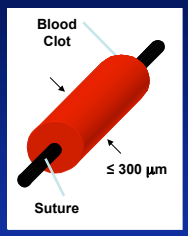
Thrombolytic Efficacy of rt-PA loaded ELIP

1. **To what degree is the lytic efficacy of rt-PA changed when the drug is encapsulated?**
2. **Can 120-kHz pulsed ultrasound be used concurrently with rt-PA-loaded ELIP to enhance thrombolysis?**

Shaw GJ *et al. Thromb Res.* 123:528-536 (2009IP)

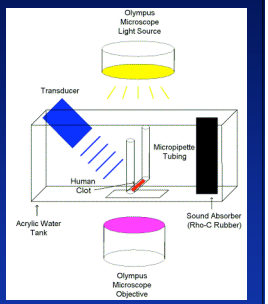
Methods

- **Human whole blood clots**
  - **Venipuncture of 22 volunteers**
  - **7-0 Silk sutures in 20 µl glass tubes**
  - **6-8 µl clots**
  - **Clot Width (W_C): 238.5 ± 34.6 µm (215 clots)**



Experimental Set-up

- **Water tank at 37°C**
- **Human fresh frozen plasma (hFFP)**
- **Inverted microscope and CCD camera**
- **Field of view: 260 µm x 340 µm**
- **Entire width of clot in field of view**

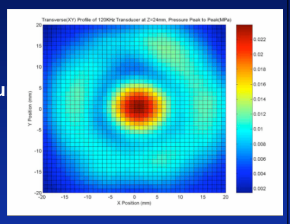


Methods

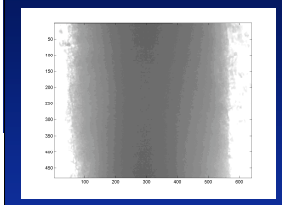
- **T-ELIP Formulation**
  - **DPPC:DOPC:DPPG:Chol (46:24:24:6 molar ratio)**
  - **Reconstituted using supersaturated de-ionized water**
- **rt-PA Concentration:**
  - **Free rt-PA added to hFFP: $[rt\text{-}PA]_{Free}$ = 3.15 µg/ml**
  - **rt-PA-loaded ELIP (t-ELIP), added to hFFP: $[rt\text{-}PA]_{t\text{-}ELIP}$ = 3.15 µg/ml**

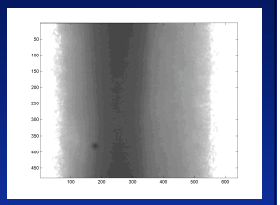
Methods

- Ultrasound Calibration
  - 120 kHz, 0.35 MPa_{p-p}
  - PRF = 1667 Hz
  - DC = 50% (36 cycles)
- Protocol: 30 min exposu to:
  1) Free rt-PA alone
  2) t-ELIP alone
  3) Free rt-PA + US
  4) t-ELIP + US



Time-lapse Thrombolysis



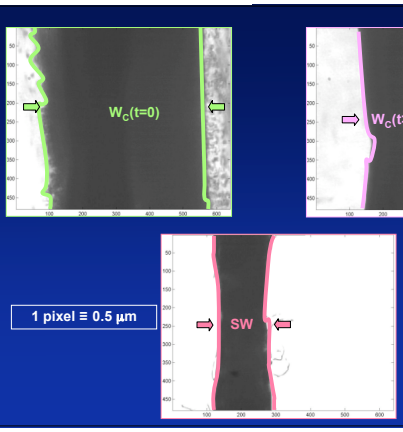


t-ELIP Alone — 28 min

US & t-ELIP — 30 min

1 frame of movie ≡ 10 seconds of data

1 second of movie ≡ 1 minute of data



1 pixel ≡ 0.5 µm

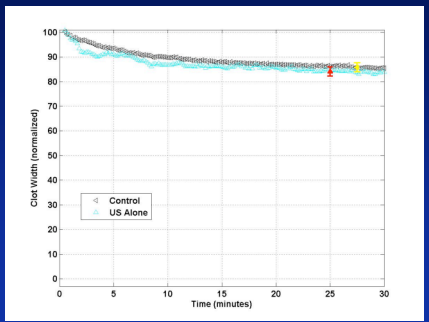
Clot Width vs. Time

- The clot width (W_C) is corrected for suture width (SW) and normalized (W_{CN})

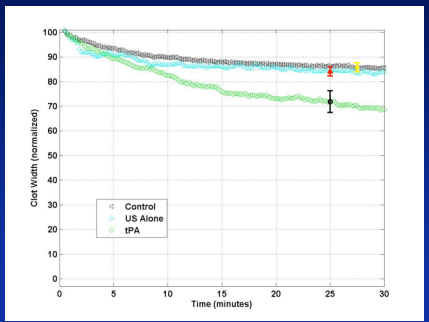
$$W_{CN}(t) \equiv \frac{W_{Corrected}(t)}{W_{Corrected}(0)} = \frac{W_C(t) - SW}{W_C(0) - SW}$$

Average SW: 95 ± 15 mm

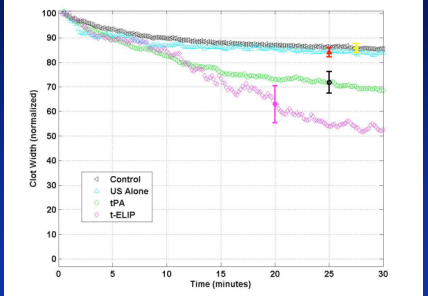
Clot Width vs. Time



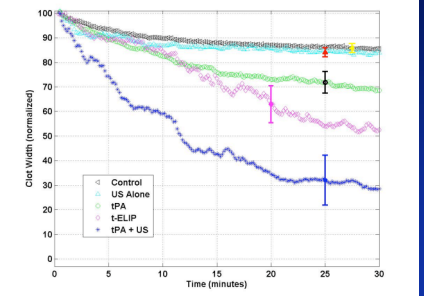
Clot Width vs. Time



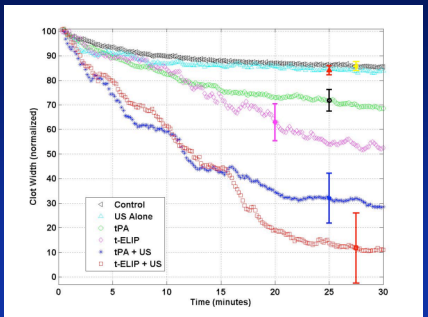
Clot Width vs. Time



Clot Width vs. Time



Clot Width vs. Time



Potential Mechanisms for Thrombolytic Enhancement with t-ELIP

- Better targeting than free rt-PA
  - Fibrin binding for t-ELIP is twice that of free rt-PA (Tiukinhoy-Laing et al., *J Drug Target*, 15:109-114, 2007)
- Less scavenging of rt-PA
- Nucleation of stable cavitation with encapsulated bubbles
  - (Datta et al., *Ultrasound Med Biol*, 32:1257-67, 2006)

Ultrasound-assisted thrombolysis for stroke therapy

Christy K. Holland, Kathryn E. Hitchcock, Nikolas Ivancevich, Kevin J. Haworth, Danielle N. Caudell, Deborah Vela, Jonathan T. Sutton, Gail J. Pyne-Geithman
Biomedical Engineering, University of Cincinnati
Cincinnati, Ohio, USA

Stroke, In Press 2010

Better Thrombus Break-up with Bubbles

- Ultrasound (US) enhances rt-PA thrombolysis[1] via stable cavitation[2]
  - Ultrasound-driven bubble activity
- One clinical study demonstrated increased intracerebral hemorrhage [3]
- Later modeling showed peak negative pressures > 1.0 MPa, standing waves [4]

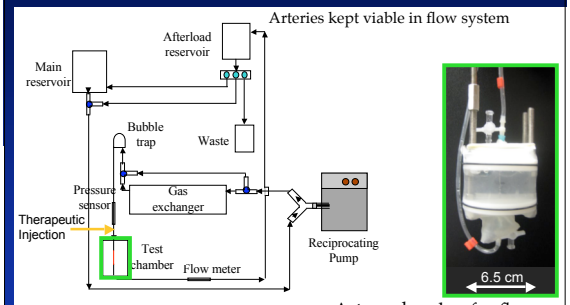
Holland *et al. Thromb Res* 2008 Datta *et al. Ultrasound Med Biol* 2006, 2008
Daffertshofer *et al. Stroke* 1995 Baron *et al. Ultrasound Med Biol* 2009

Ultrasound-enhanced clot lysis *ex vivo*

- *Research Question*: If stable cavitation is maximized, is thrombolytic enhancement also improved?
- Whole-blood clots injected into excised living porcine carotid arteries[5]
  - Protocol approved by IACUC at U. of Cincinnati
- Mounted in flow system and perfused with porcine plasma
  - Physiologic pH, pressure, temperature
  - Oxygen 23.3 ± 1.5 mg/L
  - Flow 2.7 +/- 1.8 mL/min to represent ischemia
- Arteries and clots examined histologically after treatment

Hitchcock et al., In press 2010

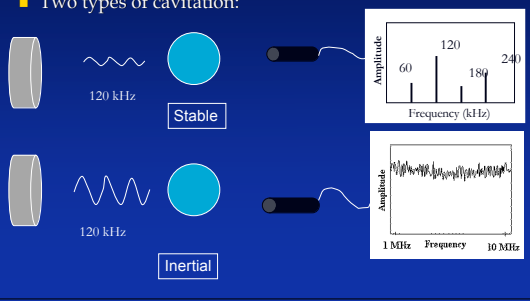
Flow system

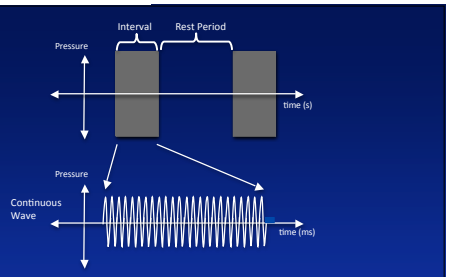


Artery chamber for flow and ultrasound coupling

Cavitation Detection

- Cavitation is the formation and collapse of gaseous and vapor bubbles in a liquid due to acoustic pressure field
- Two types of cavitation:





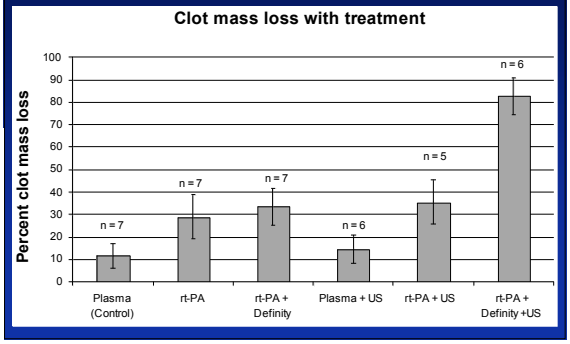
- US Parameters chosen to maximize stable cavitation
- Intermittent US, 0.44 MPa peak-to-peak amplitude
- Delivered in intervals of 8.5 s on 19.5 s off
  - Quiescent intervals permit influx of fresh Definity®

Number of clots in each treatment group:

	Plasma	Plasma and rt-PA	Plasma and rt-PA with Definity®
No ultrasound	7	7	7
With ultrasound	6	5	6

- 1.25 cm of 1.9 ± 0.2 cm clot inside US beam (66%)

Results: US-enhanced clot lysis *ex vivo*



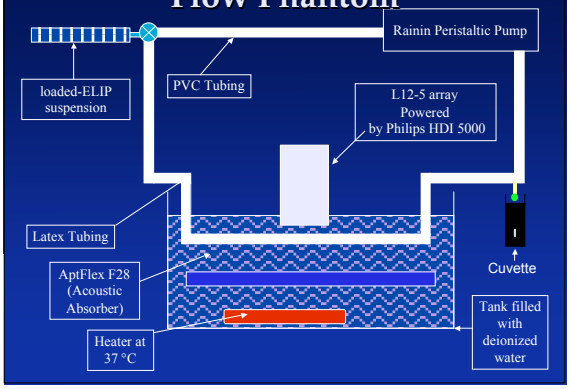
Results: US-enhanced clot lysis *ex vivo*

Histology:

- Loss of endothelium in ischemic environment
  - 64% ± 28% of observed endothelial segments showed some loss
- Edema in 10% of tunica media
- Slight anti-t-PA staining in artery wall
- No relationship to treatment type
  - ANOVA: $F(6, 26) = 1.47$, $p = 0.23$

Can stable cavitation be sustained with a clinical ultrasound scanner using an ELIP infusion scheme?

Flow Phantom



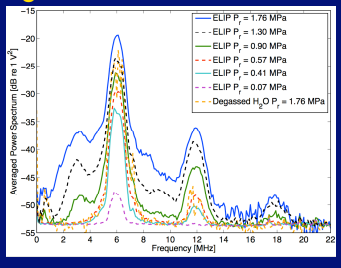
Experimental details…

- ELIP 0.02 mg/ml lipid in 0.5% BSA
- 5 ml/min pulsatile flow
- 6-MHz Color Doppler pulse from L12-5 (Philips HDI 5000)
- PRF = 700 Hz
- FR = 12 Hz
- 10-MHz PCD confocally aligned with first line of Color Doppler pulses

PCD signal processing

- PCD signals recorded and processed in MATLAB
- Computed power spectrum averaged over 20 s
- Subtracted averaged electronic noise in high frequency band beyond PCD and L12-5 -6dB bandwidth

Can stable cavitation be sustained with a clinical ultrasound scanner using an ELIP infusion scheme?



YES!

Acknowledgements

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NIH/NINDS R01-NS047603
NIH/NHLBI R01-HL074002
NIH/NHLBI R01-HL059586

Acknowledgements