Emerging Techniques: Focused Ultrasound

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Focused Ultrasound Foundation

UM Biosciences Initiative

Patents

US-10,322,184; US-10,335,368; US-10,709,812

National Institute of Arthritis and Musculoskeletal and Skin Diseases

National Heart, Lung, and Blood Institute

Focused Ultrasound Foundation

Biosciences University of Michigan

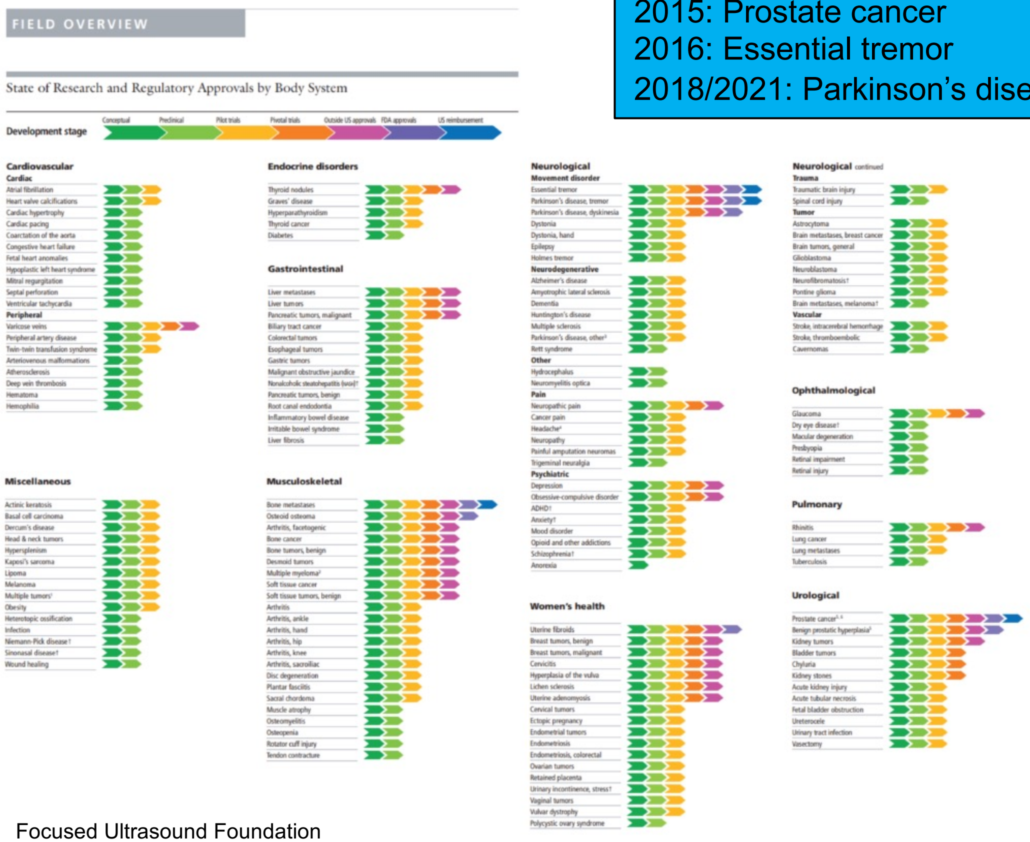
Focused ultrasound is a rapidly growing technology that is revolutionizing medical therapies

The benefits of FUS

- Non-ionizing
- Non-invasive
- Spatiotemporally controlled
- Translational

FUS, alone or in combination with administered agents, can provide therapeutic benefits in many applications

US FDA approval
2004: Uterine fibroids
2012: Bone metastases
2015: Prostate cancer
2016: Essential tremor
2018/2021: Parkinson’s disease



Focused Ultrasound Foundation

Outline

- Basics of FUS
- Applications of FUS in orthopedics and regenerative medicine
  - Clinical
  - Pre-clinical

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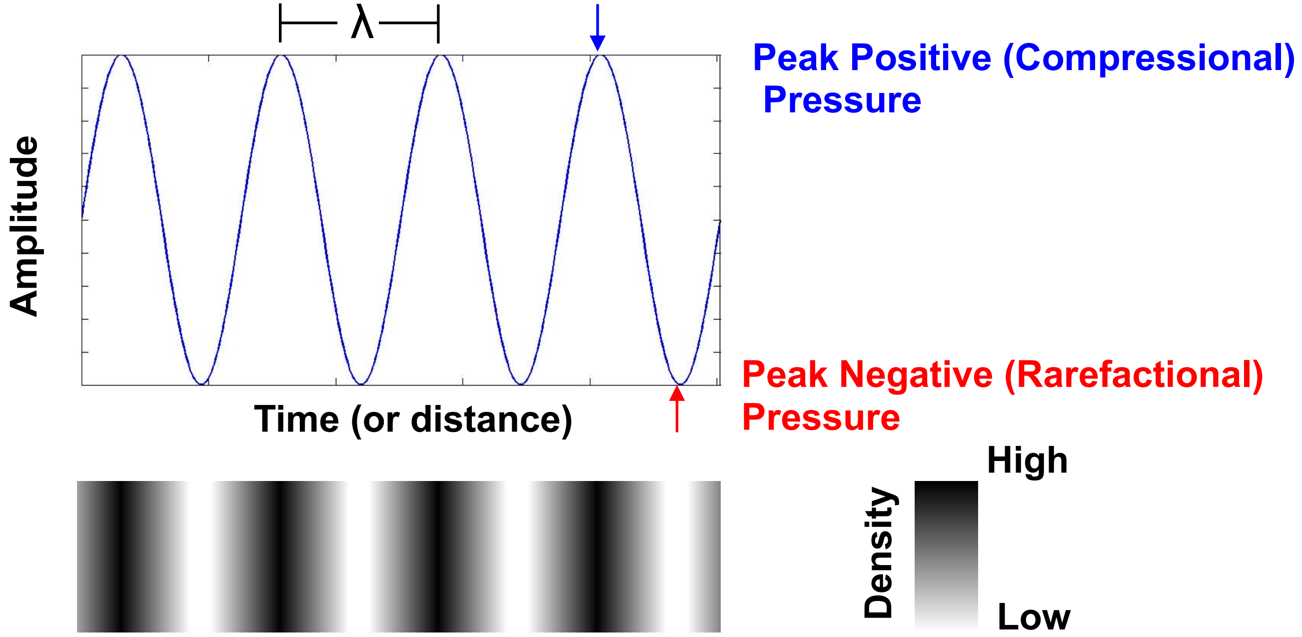
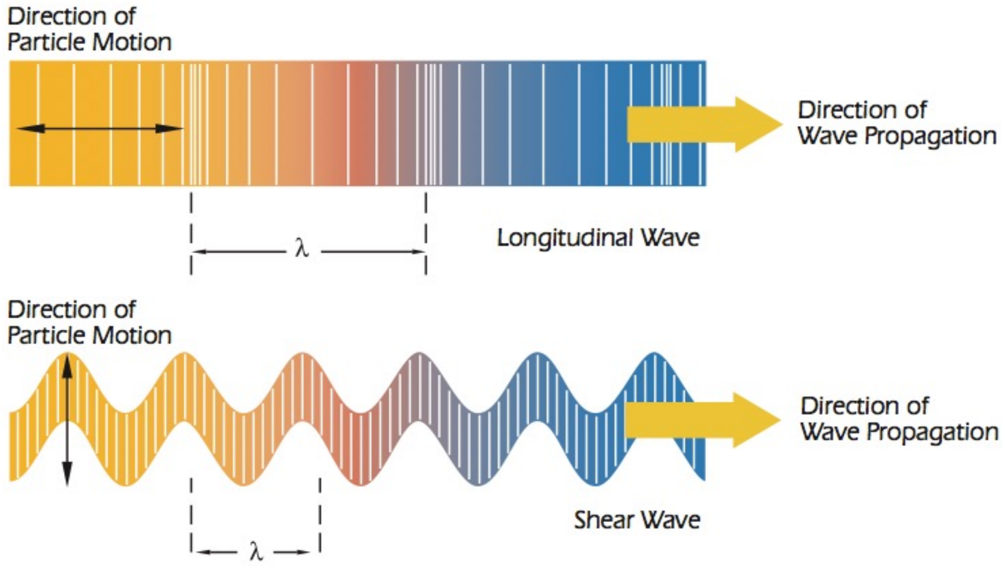
The Physics of (Ultra)sound

Sound is a type of mechanical wave

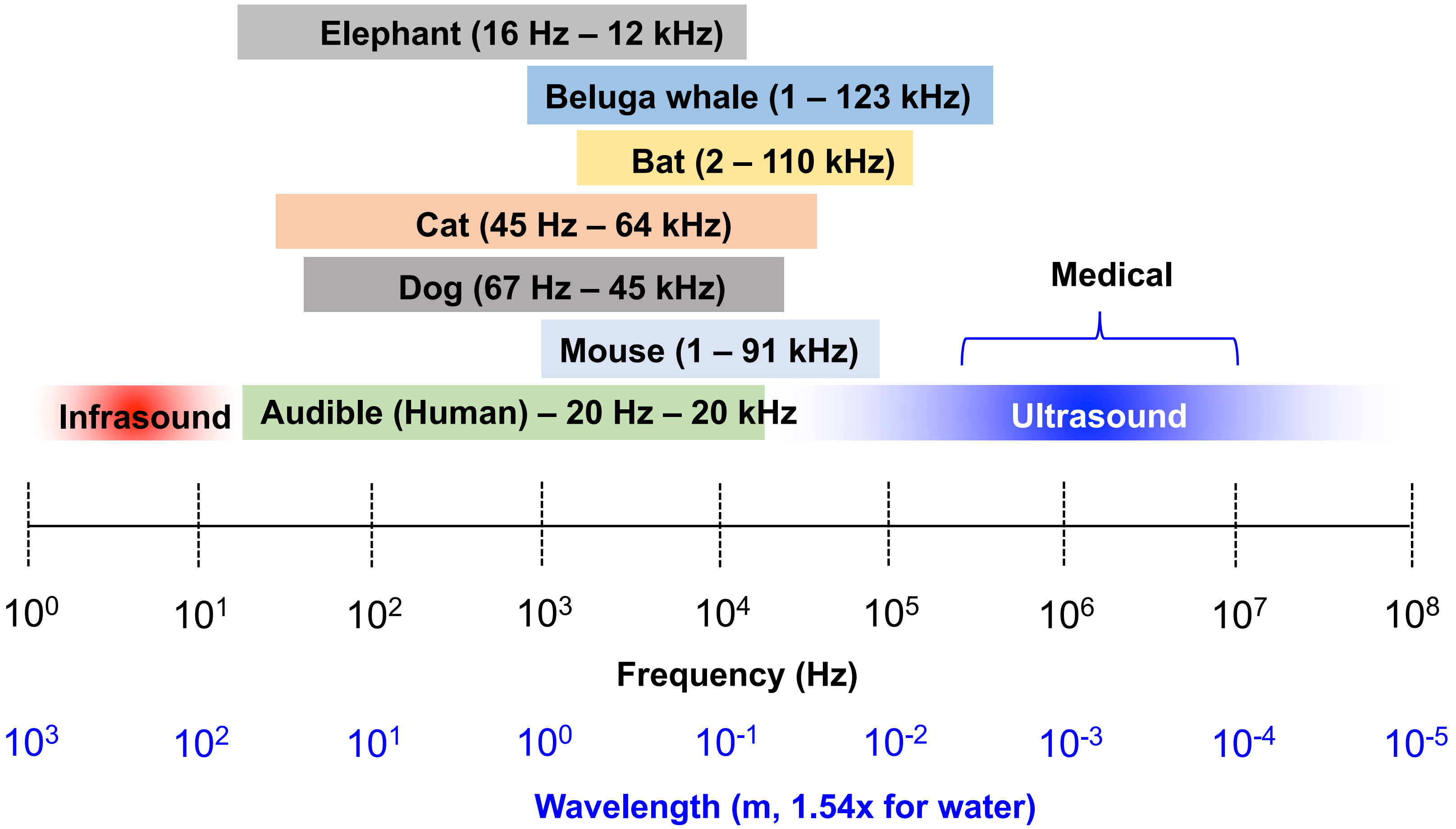
- Oscillation in pressure, stress, particle displacement, particle velocity, etc., propagated in a medium with internal forces (e.g., elastic or viscous), or the superposition of such propagated oscillation (ANSI/ASA S1.1-2013)
- Unlike light, sound does NOT propagate through a vacuum





www.olympus-ims.com

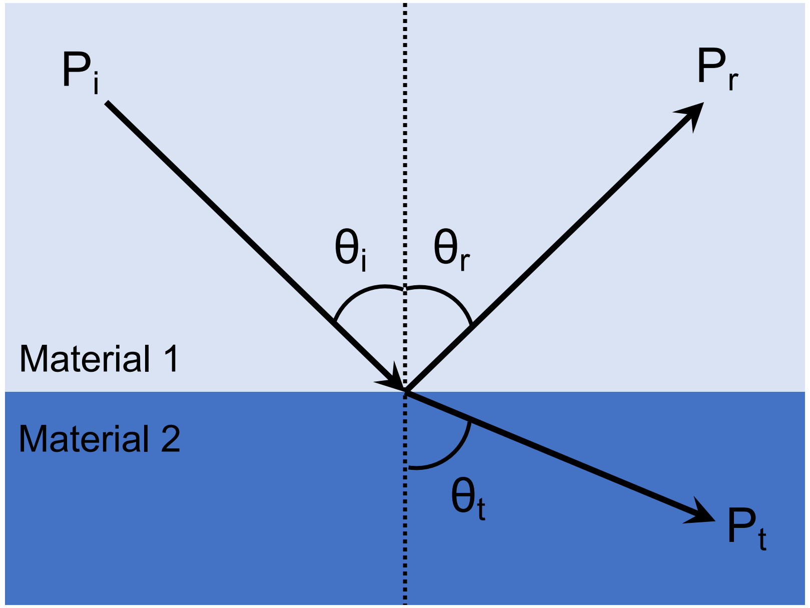
The Range of Sound Frequencies



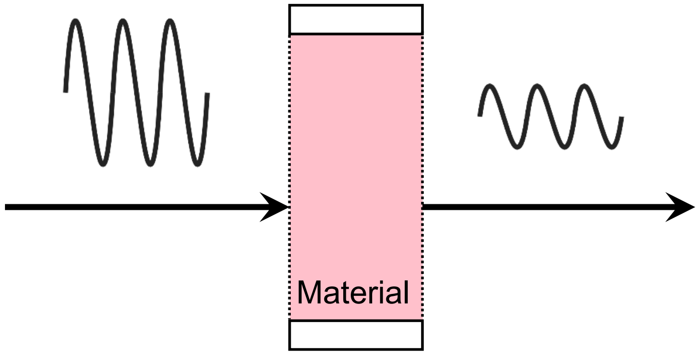
http://www.lsu.edu/deafness/HearingRange.html

Ultrasound propagation is dependent on material properties

Reflection and Transmission



Attenuation



	Attenuation (dB/cm/MHz)
Water	0.02
0.5% (w/v) fibrin	0.03
1.0% (w/v) fibrin	0.04
2.0% (w/v) fibrin	0.07
Soft tissue (average)	0.54
Muscle	1.09
Bone, cortical	6.90
Bone, trabecular	9.94

$Z = \rho c$
Z = acoustic impedance
ρ = material density
C = material sound speed

$\alpha = \alpha_S + \Delta\alpha_V + \alpha_V$
α = acoustic attenuation
α_S = scattering term
α_V = absorption term

Diagnostic vs Therapeutic Ultrasound

Diagnostic ultrasound

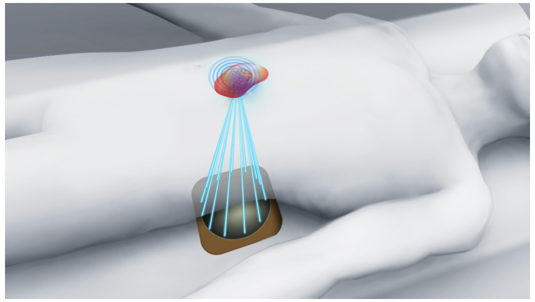
- “Look” only
- Primary goal: **NOT** generate bioeffects

GE Healthcare

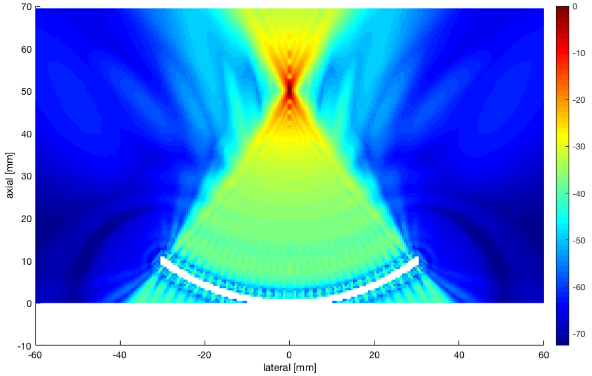
Therapeutic ultrasound

- Primary goal: generate bioeffects
- Bioeffects can be mechanical and/or thermal

Sonic Concepts

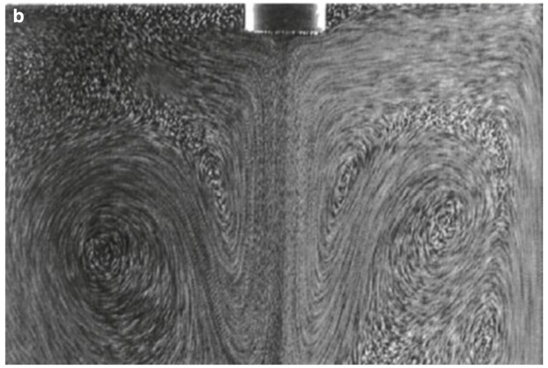


Sonalleve



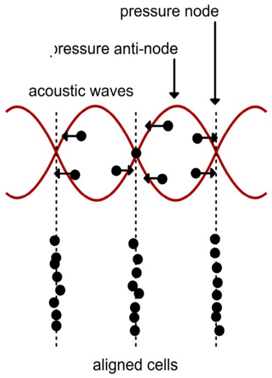
Ultrasound-induced phenomena can generate multiple bioeffects

Radiation Force



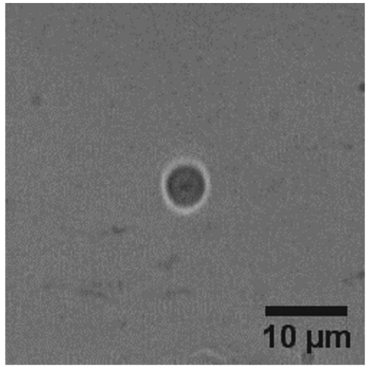
Nowicki et al. *Eur J Ultrasound* 1998

Surface Waves



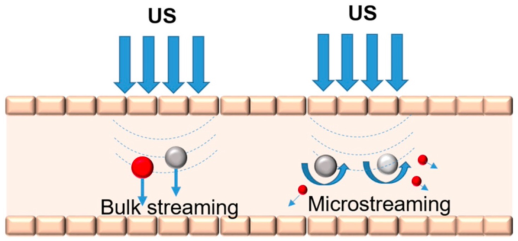
Naseer et al. *Biofabrication* 2017

Acoustic Droplet Vaporization



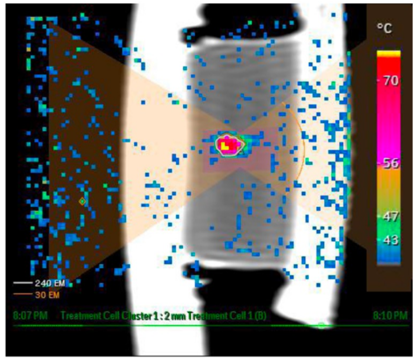
Sheeram (unpublished)

Acoustic Streaming



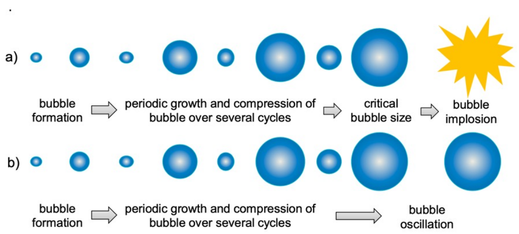
Duan et al. *Theranostics* 2020

Heating



Kripfgans et al. *J Acoust Soc Am* 2014

Cavitation



Izadifar et al. *J Med Biol Eng* 2019

<u>Bioeffects are dependent on</u>

- Frequency
- Amplitude/Pressure
- Pulse duration
- Pulse repetition frequency
- Duty cycle
- Exposure location
- Tissue pathology
- Exogenous agents
- …

$\left.\begin{array}{l}\text{Frequency}\\ \text{Amplitude/Pressure}\\ \text{Pulse duration}\\ \text{Pulse repetition frequency}\\ \text{Duty cycle}\end{array}\right\}$ Intensity (“Acoustic Dose”)

Outline

- Basics of FUS
- Applications of FUS in orthopedics and regenerative medicine
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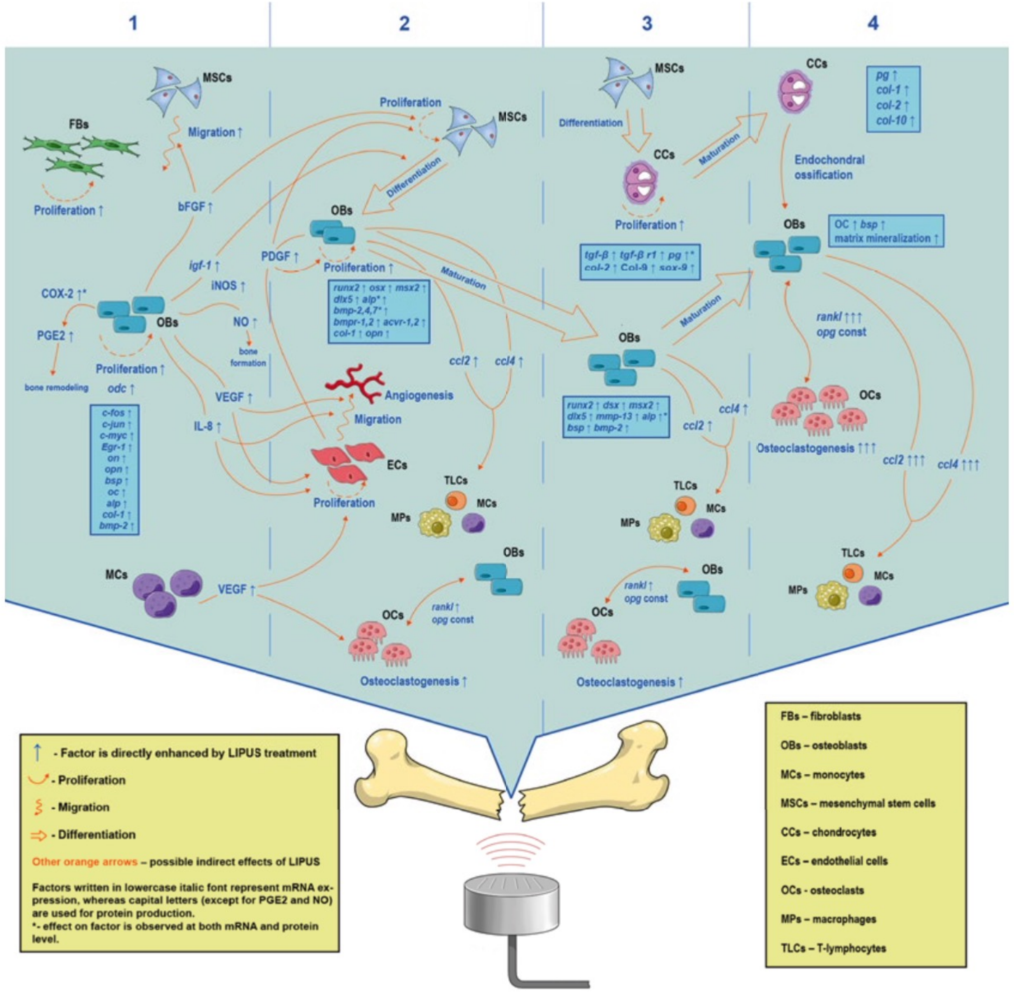
Low intensity pulsed ultrasound (LIPUS) has been clinically used in bone repair

- Uses diagnostic level intensities
- Stimulates mechanotransductive pathways (e.g., MAPK, integrins, etc.)
- Received US FDA approval in 1994 for radius and tibial fractures; in 2000, non-unions (not skull/vertebrae)
- Clinical benefits are “muddied”

Exogen (Smith & Nephew, Bioventus)

<u>Parameters</u>
1.5 MHz, 1 kHz PRF, 20% DC
30 mW/cm^2
20 min per day



Escoffre and Boukaz (eds.) *Therapeutic Ultrasound* 2016

Outline

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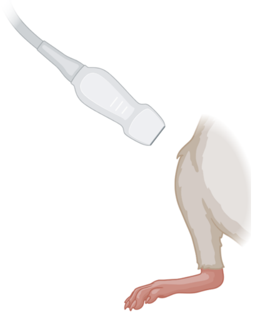
FUS enhances targeting of exogenous MSCs to skeletal muscle

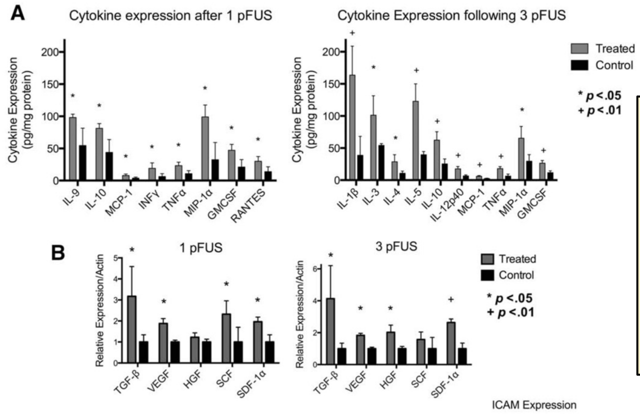
Problem: Poor localization of MSCs after IV injection

Approach: Use FUS to transiently increase levels of chemoattractants in target tissue

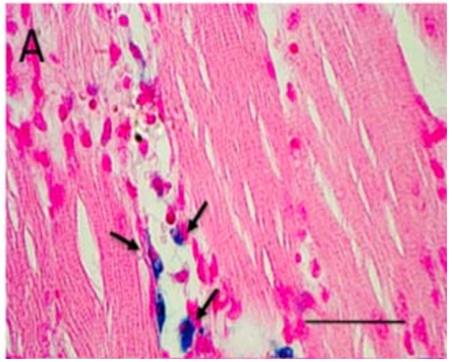
Sonoblate 500
1 MHz, 5% DC
I_{SATA}: 133 W/cm^2

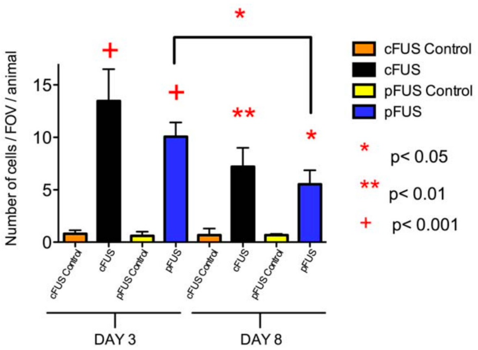


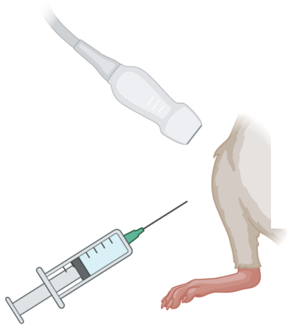
C3H mice (hamstring)



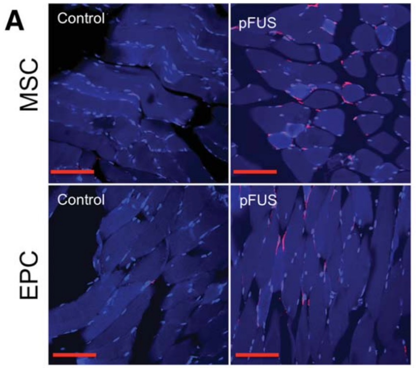
FUS effects in muscle
↑ cytokines
↑ growth factors
↑ M2 macrophages
No tissue damage



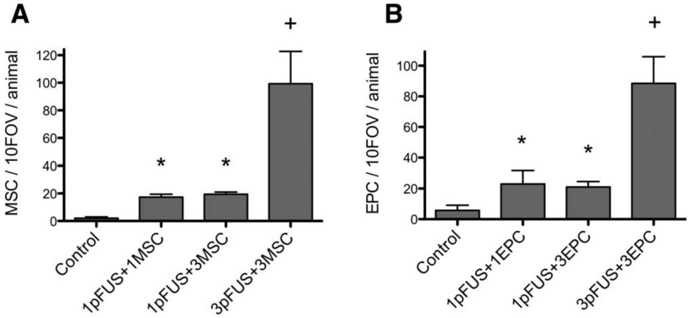




10^6 MSCs or
EPCs injected IV
2-3 after FUS



FUS ↑ cell homing in a dose dependent manner



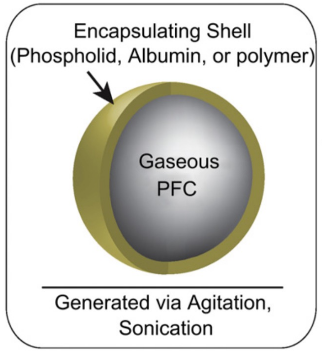
[1] Burks et al. *PLoS One* 2011
[2] Burks et al. *Stem Cells* 2013

Sonoporation increases BMP2 plasmid uptake and bone formation

Problem: Safe and effective delivery of BMP2 is challenging

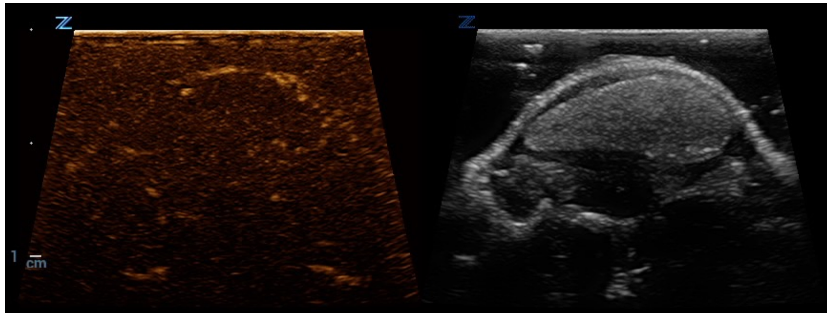
Approach: Use FUS and microbubbles to locally enhance BMP2 plasmid delivery

Microbubble Contrast Agent



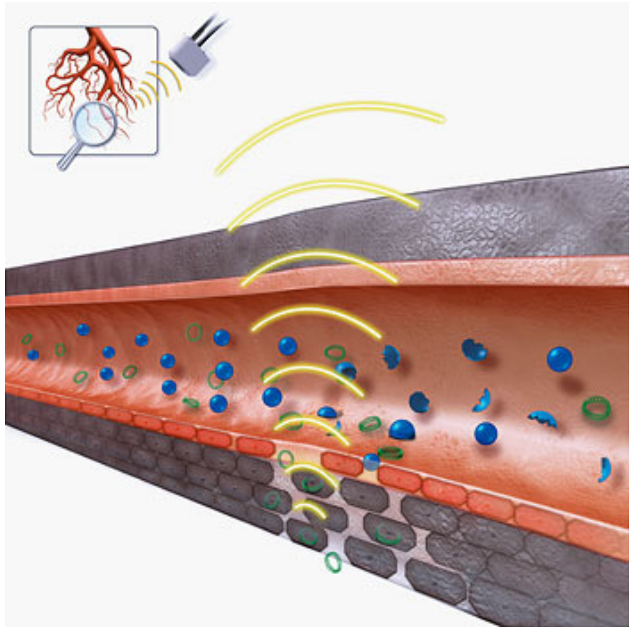
Sheeran et al.
Biomaterials 2012

Contrast Enhanced Ultrasound



CEUS in SKH-1 Elite mouse (*unpublished*)

Sonoporation



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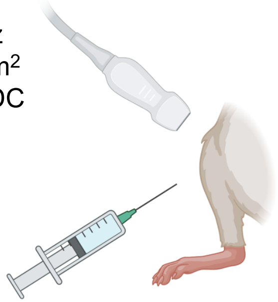
1 MHz
4 w/cm^2
50% DC
5 min



Local co-injection of Microbubbles + pDNA$_{BMP2}$

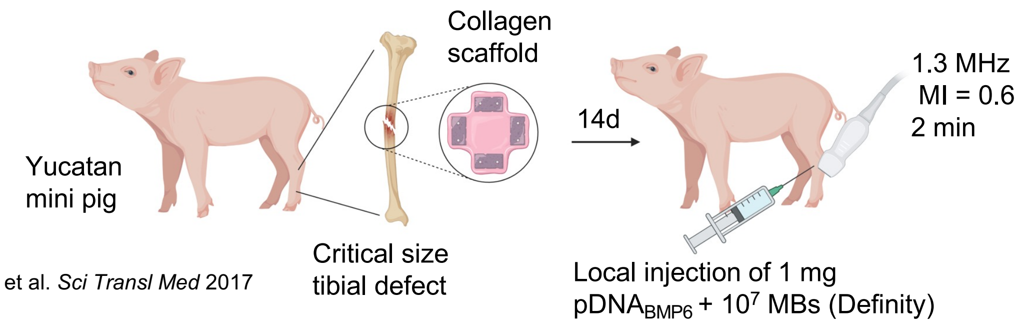
ICR mice (gastrocnemius)

Osawa et al. *J Gene Med* 2009

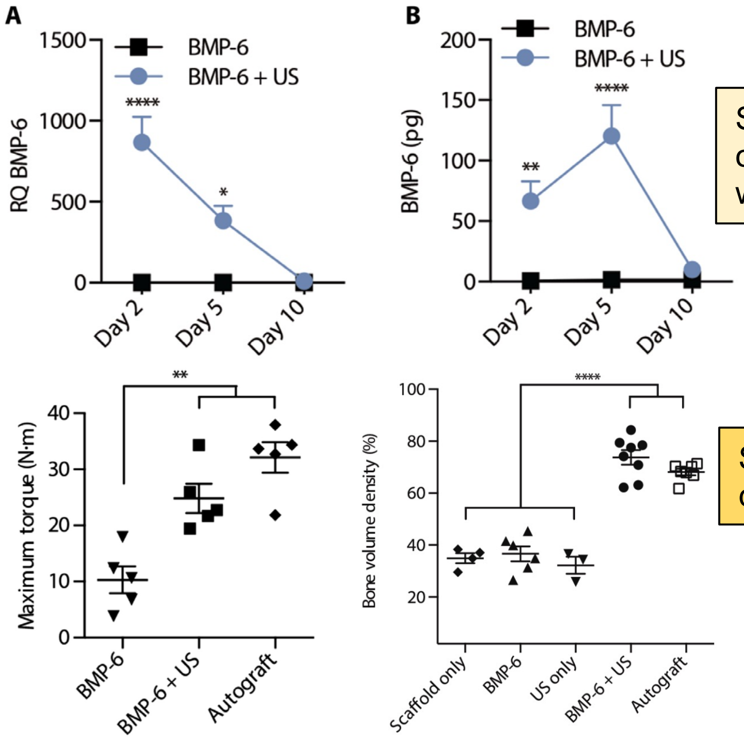
D7 D14 D21 D21

pDNA$_{BMP2}$ + microbubbles + FUS | pDNA$_{BMP2}$

Sonoporation of endogenous MSCs improves fracture healing and ligament reconstruction

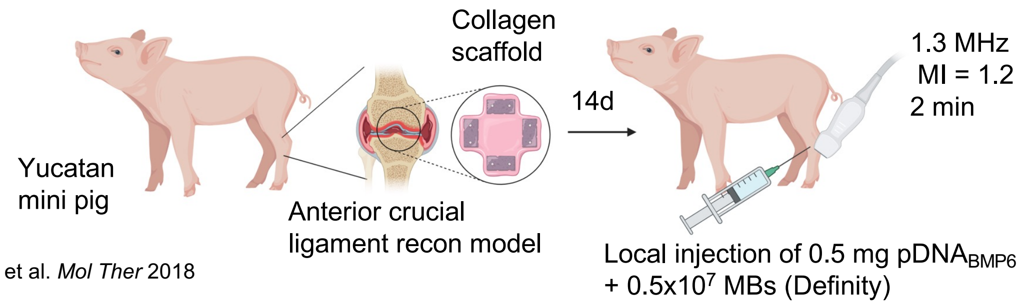


Bez et al. *Sci Transl Med* 2017

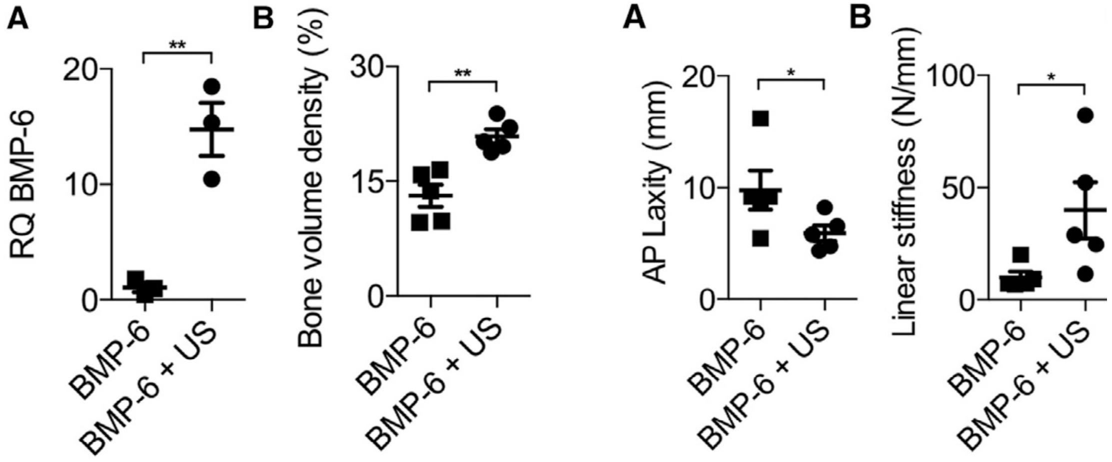


Sonoporation transfected 40% of cells at fracture site (80% of which were MSCs)

Sonoporation caused complete healing of fracture



Bez et al. *Mol Ther* 2018

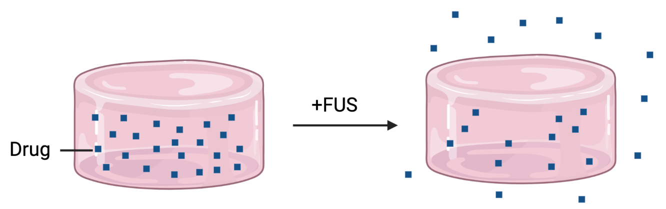


Sonoporation enhanced BMP6 delivery and osteointegration

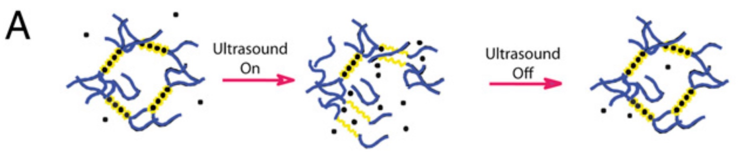
FUS enables on-demand control of drug release from hydrogels

<u>Problem</u>: The ability to spatiotemporally control drug release from hydrogels is very limited

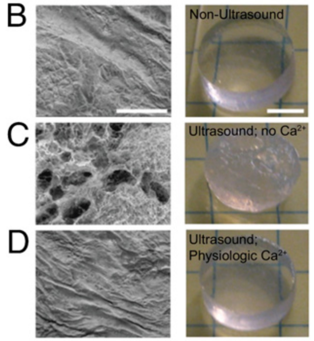
<u>Approach</u>: Develop drug-loaded hydrogels that can be modulated using FUS

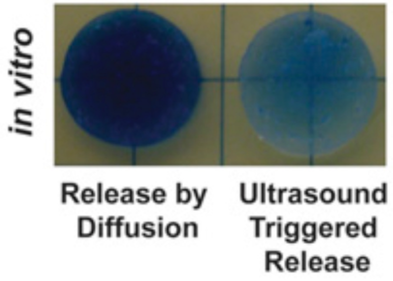


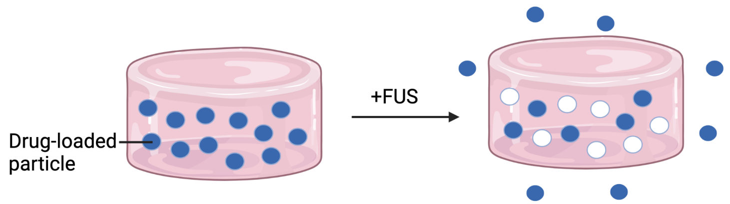
Hydrogel: alginate
Drugs: mitoxantrone, SDF-1α, pDNA

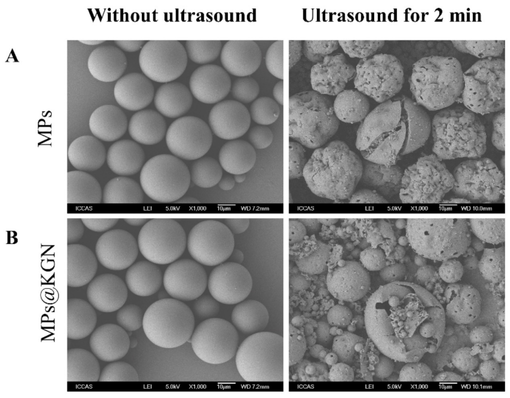


Huebsch et al. *Proc Natl Acad Sci* 2014



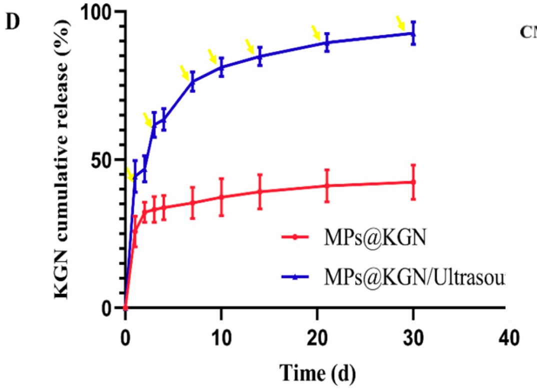




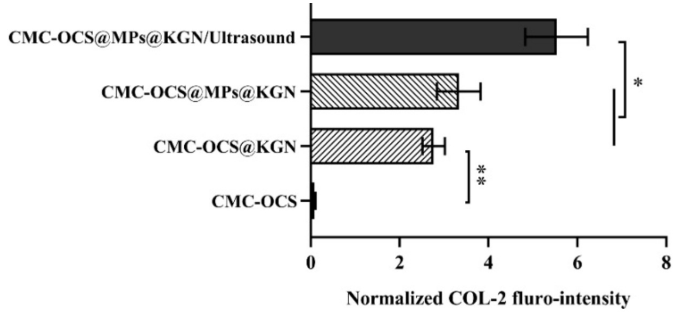


Yuan et al. *Pharmaceutics* 2021

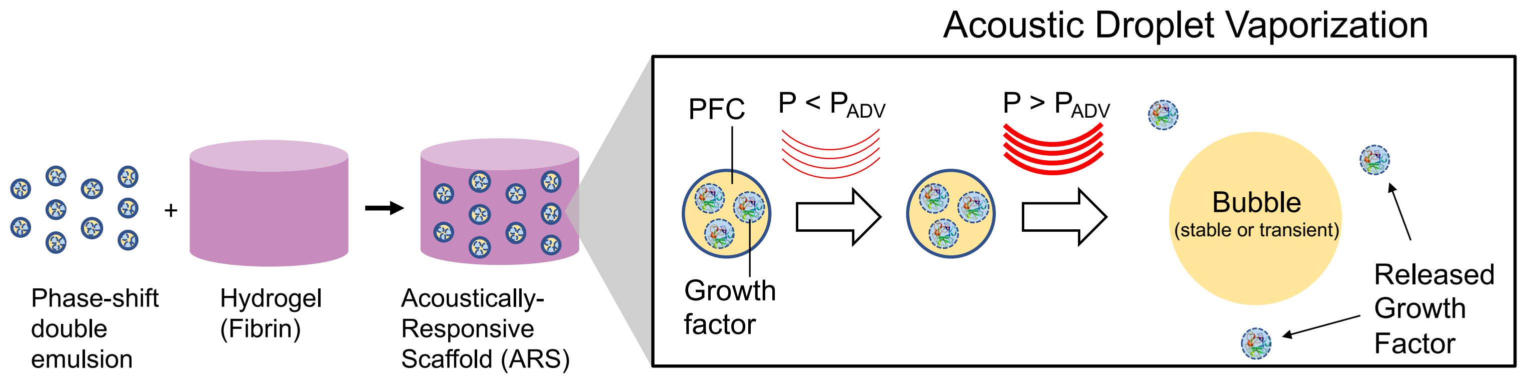
Hydrogel: chondroitin sulfate
Particles: PLGA
Drug: kartogenin

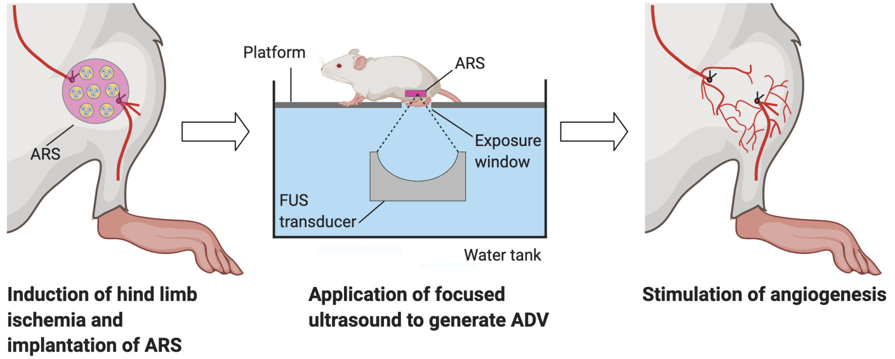


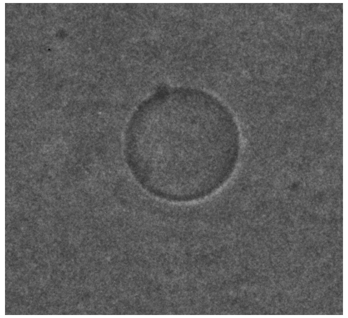
FUS ↑ chondrogenic differentiation

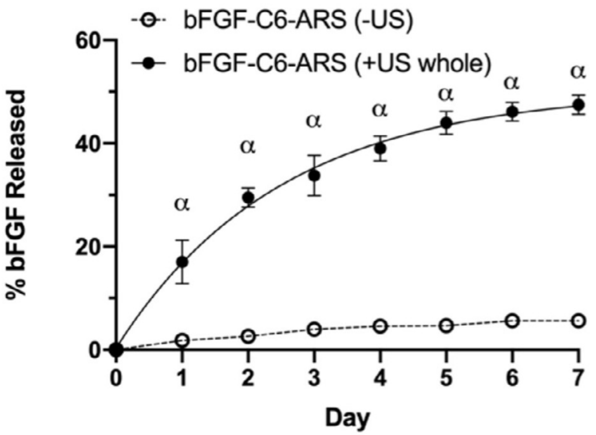


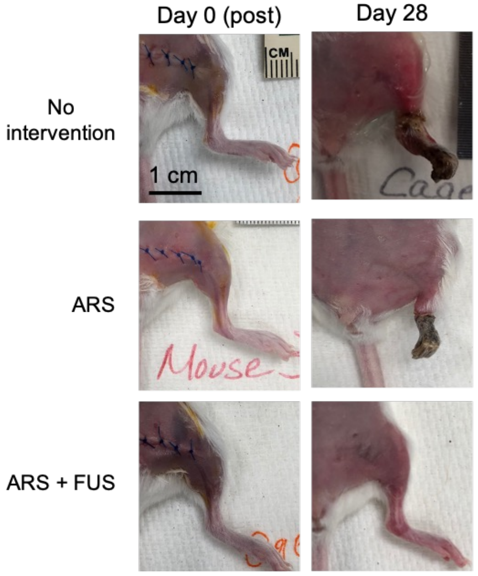
FUS-mediated growth factor release from a composite hydrogel stimulates angiogenesis

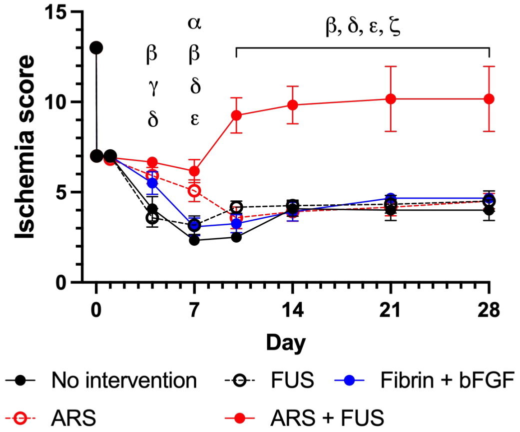










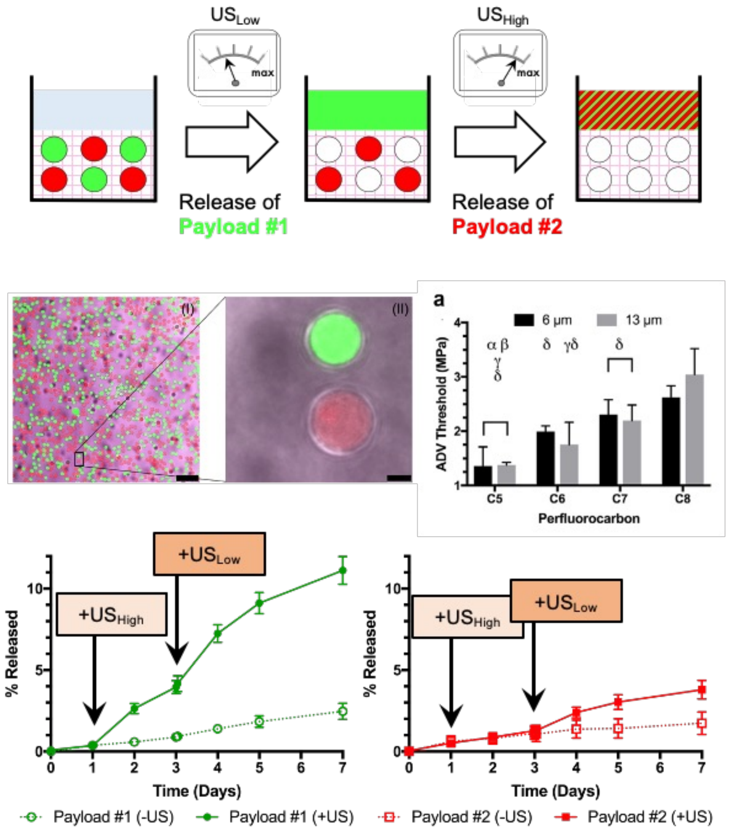


FUS mediated delivery of bFGF
↑ perfusion
↑ angiogenesis
↓ tissue necrosis
↓ tissue fibrosis

[1] Lu et al. *Acta Biomater* 2020
[2] Jin et al. *Pharmaceutics* 2021

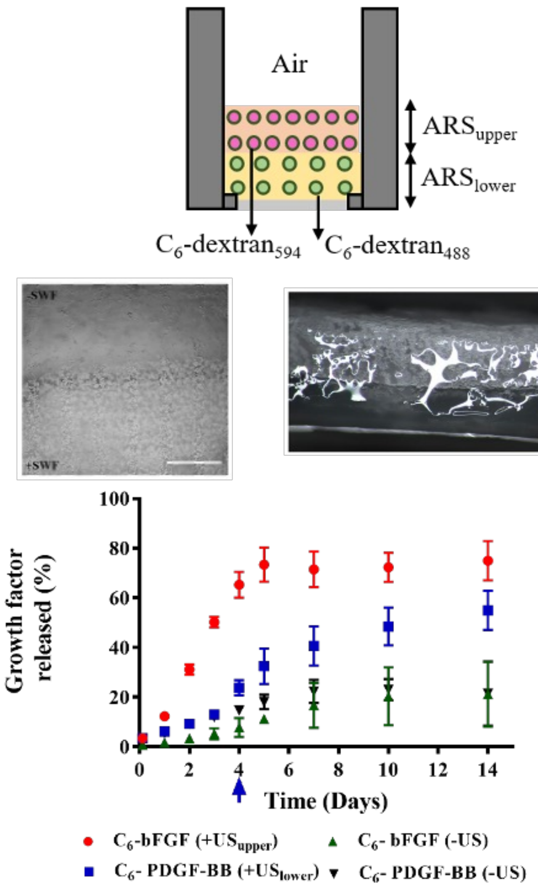
Sequential payload release using composite hydrogels and FUS

Strategy #1
Two emulsions (PFC_1, PFC_2) and pressures (P_1, P_2)



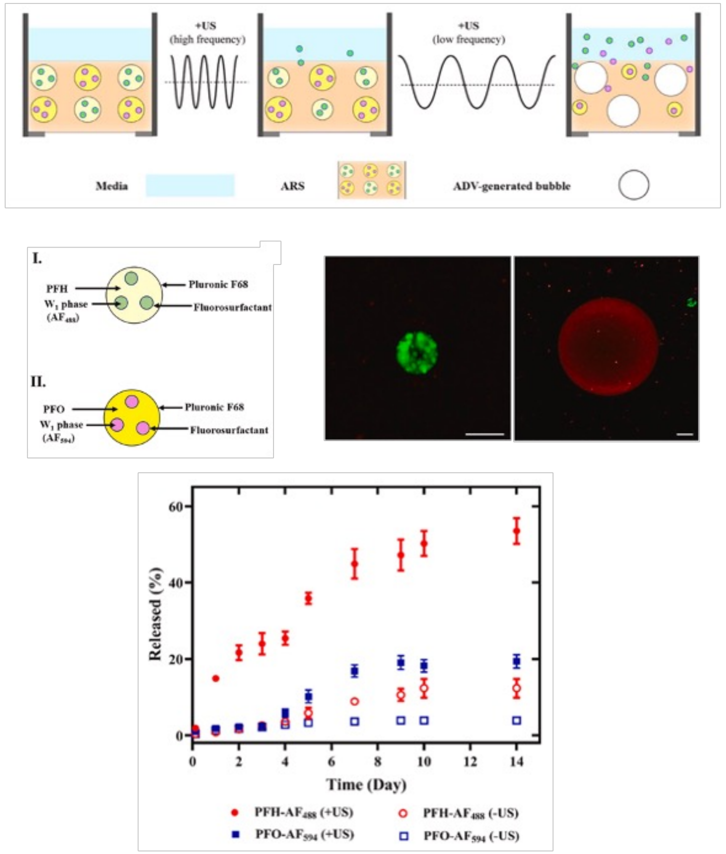
Moncion et al. *Ultrasound Med Biol* 2018

Strategy #2
Bi-layer ARSs with standing wave field



Aliabouzar et al. *Ultrason Sonochem* 2020

Strategy #3
Two emulsions (PFC_1, PFC_2) and frequencies (F_1, F_2)

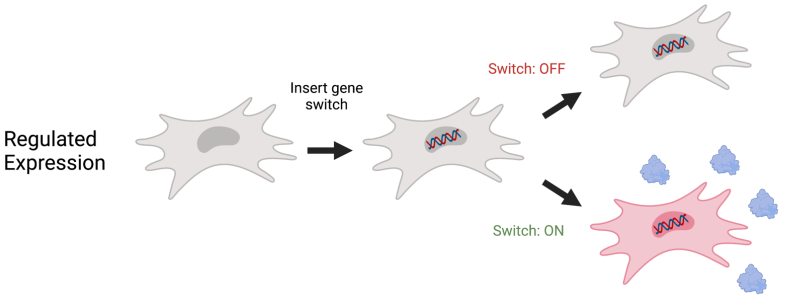


Aliabouzar et al. *Ultrason Sonochem* 2021

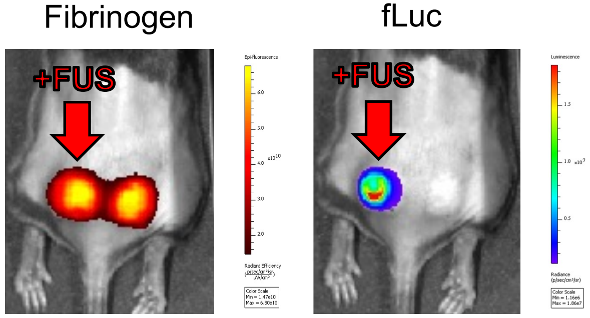
FUS-generated hyperthermia enables spatiotemporally controlled transgene expression

<u>Problem</u>: The ability to spatiotemporally control drug release from hydrogels is very limited

<u>Approach</u>: Use FUS to thermally activate cells containing a heat responsive gene switch



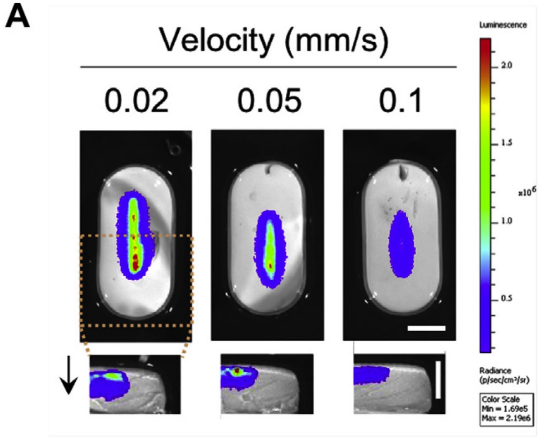
Heat shock	-	+	-	+
Ligand (i.e., dimerizer)	-	-	+	+
Transgene Expression	-	-	-	+

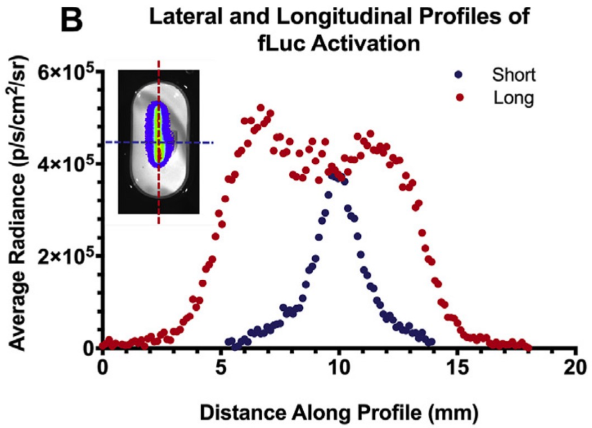


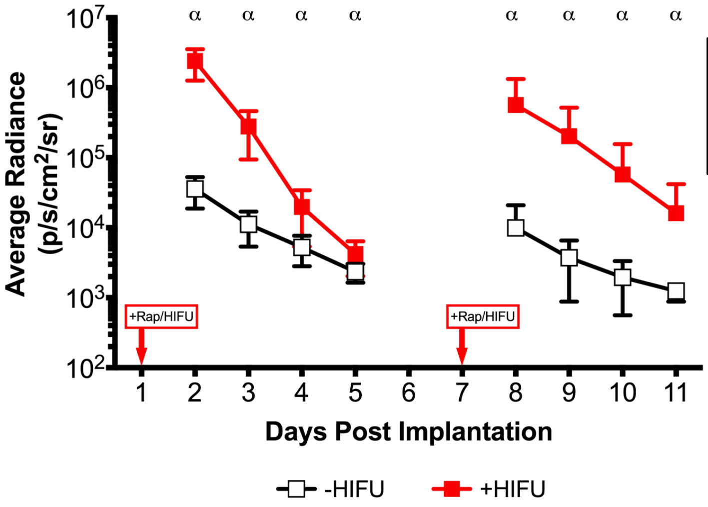
C3H mice

Hydrogel: Fibrin + HA
Cells: C3H/10T1/2-fLuc
Ligand: rapamycin

<u>FUS</u>
2.5 MHz, 258 W/cm2,
CW, 2 min (single pt)





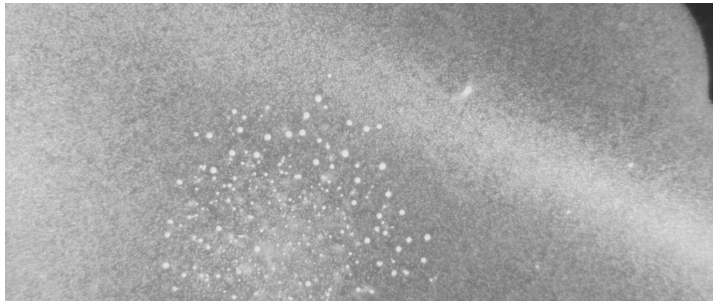


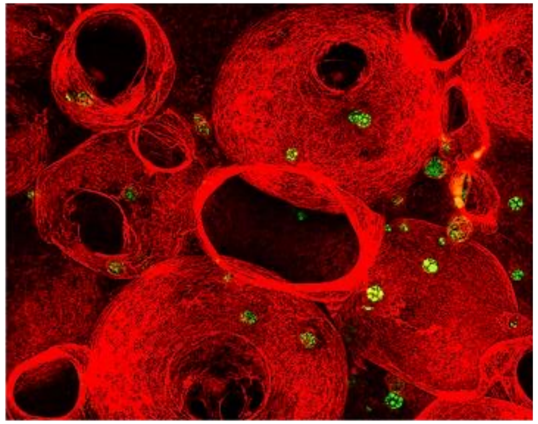
Repeated activation of transgene

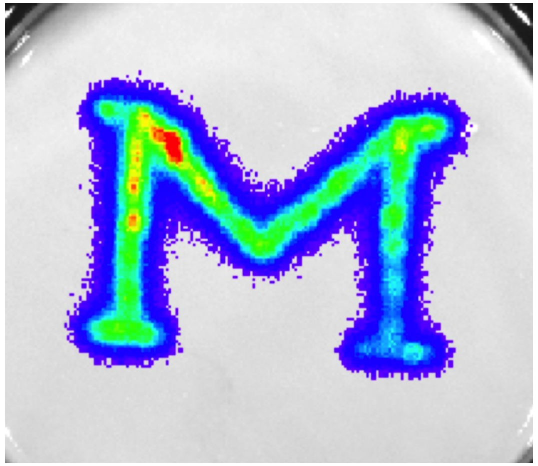
[1] Wilson et al. *Tissue Eng Pt C* 2014
[2] Moncion et al. *Biomater* 2019

Conclusions

- FUS is revolutionizing medical therapies and is well-positioned to impact orthopedics and tissue regeneration
- FUS-generated bioeffects enable non-invasive, spatiotemporally controlled modulation of in situ biology
- FUS enables highly personalized therapies that can ultimately benefit patients







Acknowledgements

<u>Fabiilli Lab</u>

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- Keith Arlotta
- Natalie Chan
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Thank you!

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