Nanotechnology and ultrasound

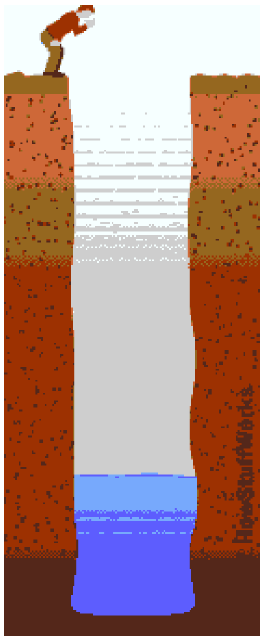
Theo Pavan

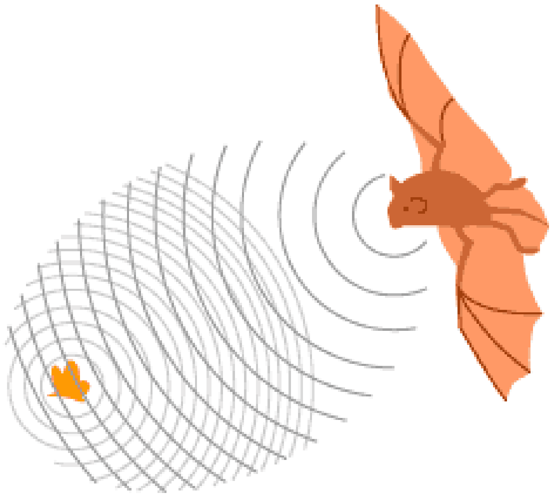
Outline

- Quick introduction about ultrasound imaging
- Contrast enhanced ultrasound with microbubbles
- Molecular images with microbubbles
- Nanobubbles
- Magneto-motive ultrasound
- Therapeutics

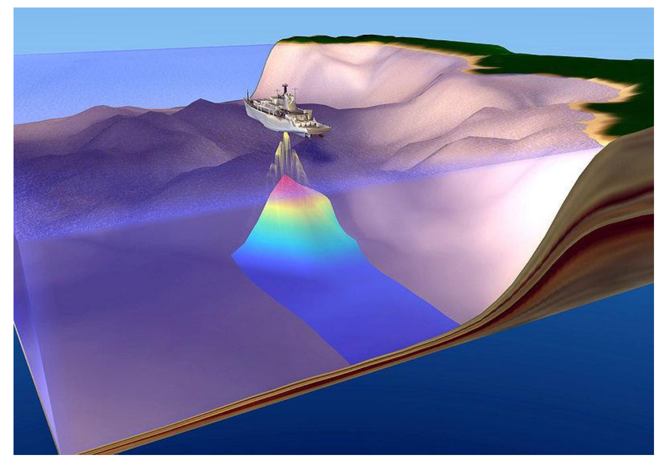
Ultrasound

Audible sound





Ultrasound ~ kHz



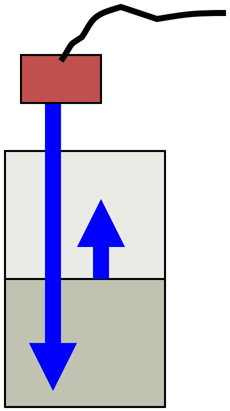
- **Medical ultrasound 1 to 15 MHz.**

Ultrasound

A ultra-sonografia, ou ecografia, é um método diagnóstico que aproveita o eco produzido pelo som para ver em tempo real as reflexões produzidas pelas estruturas e órgãos do organismo
Aparelho de ultra-som
Transdutor
Eco
Onda incidente
Freqüência de 2 até 14 Mhz
Ecografia
Os ecos gerados são interpretados através de computação gráfica. Quanto maior a freqüência, maior a resolução obtida

Impedância acústica (Z_a)

O eco só surge quando o feixe de ultrassom passa por dois meios com diferentes impedâncias.



$$\boxed{Z = \rho . v}$$

- **Z - impedância acústica**
- **ρ - densidade do meio**
- **V - velocidade do som nesse meio**

Impedância acústica

Body Tissue	Acoustic Impedance (10^6 Rayls)
Air	0.0004
Lung	0.18
Fat	1.34
Liver	1.65
Blood	1.65
Kidney	1.63
Muscle	1.71
Bone	7.8

Rayl → kg/(m².s)

Homenagem a Lord Rayleigh

Tipos de espalhamento

Vamos classificar os espalhadores em 3 classes:

Classe 1:

- Causado por concentração de uma ou mais dezenas de espalhadores por célula de resolução; **ka<<1** (produto do número de onda pelo raio do espalhador).
- É difusivo**.**
- Origem dos **speckles**. Agregados; efeitos combinados.

Classe 2:

- Causado por espalhadores com concentração de unidades por célula de resolução.
- Espalhadores dão origem a espalhamento difrativo (acho que inventei esse termo☺)**.**
- Espalhadores são independentes e distinguíveis.

Classe 3:

- Espalhadores dão origem a espalhamento especular. **ka>>1.**
- Associado aos limites de órgão e vasos calibrosos.

Tipos de espalhamento

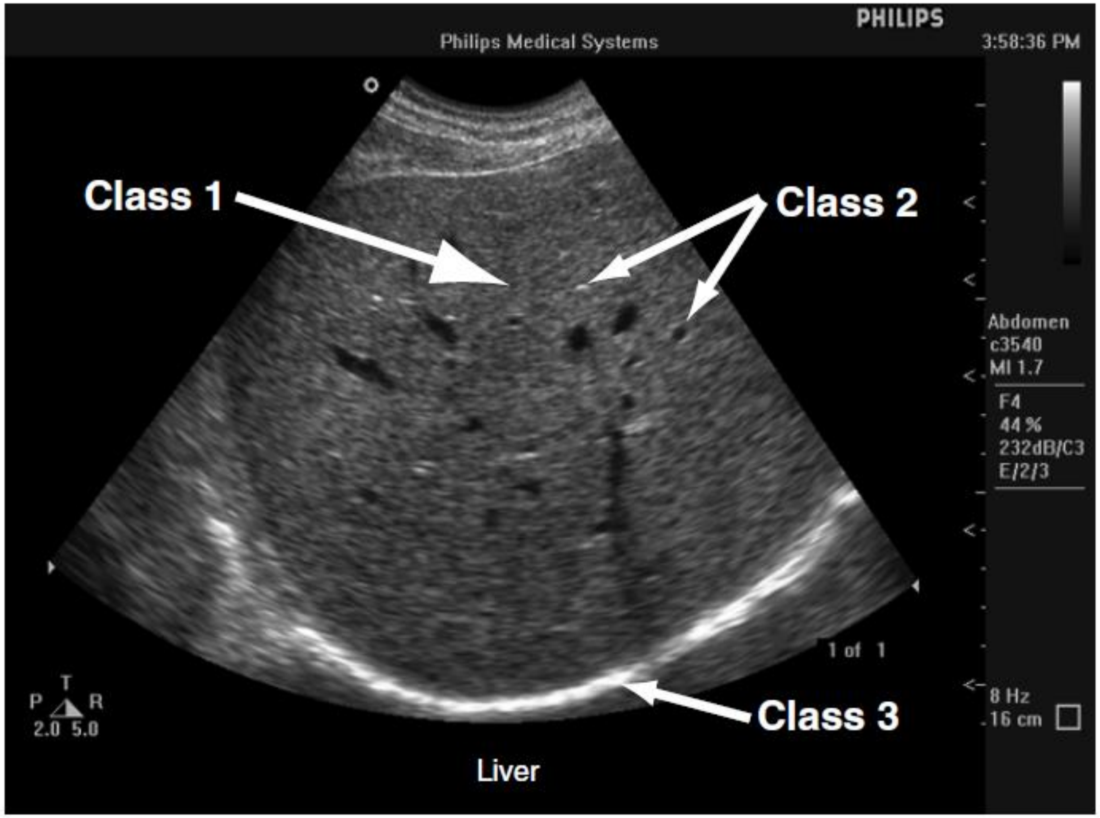


Figure 9.1 Ultrasound image of a liver showing four types of scattering effects (courtesy of Philips Medical Systems).

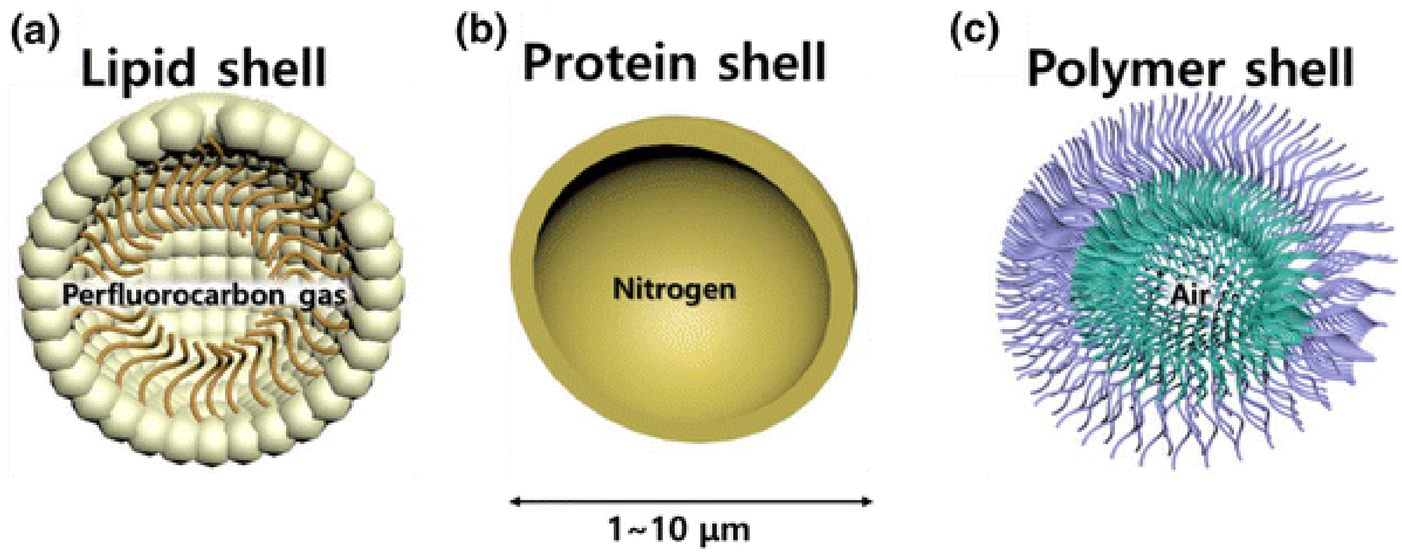
Contrast-enhanced ultrasound

- Contrast-enhanced ultrasound can be used to image blood perfusion in organs, measure blood flow rate in the heart and other organs, and for other applications.
- Commercially available contrast media are gas-filled microbubbles that are administered intravenously to the systemic circulation.
- Microbubbles have a high degree of echogenicity (the ability of an object to reflect ultrasound waves).

https://en.wikipedia.org/wiki/Contrast-enhanced_ultrasound

Microbubble

- Microbubbles generally consist of a shell that surrounds a core gas. Materials that often comprise microbubble shells include lipids, proteins, and polymers. Air, nitrogen, and perfluorocarbon are typically used as the core gas.



Biomed. Eng. Lett. (2017) 7:59–69 DOI 10.1007/s13534-017-0016-5

Microbubbles

- The microbubbles typically range from 1 to 10 μm diameter (red blood cell diameter is 8 μm).

- Permits unhindered passage from the peripheral injection site through the pulmonary vasculature with subsequent entrance into the left heart chambers and access to the systemic circulation.

Microbubble

- When a gas bubble is insonified by a US wave, it generates two kinds of responses.

Large Difference in Acoustic Impedance → **Increase Wave Scattering**

Frinking et al. Ultrasound in Med. & Biol., Vol. 26, No. 6, pp. 965–975, 2000

Microbubble

- More importantly, however, when the bubble size is much smaller than the wavelength of the US wave, it is forced into volume pulsation (for a 3-MHz US wave, the wavelength in water is 0.5 mm).

Frinking et al. Ultrasound in Med. & Biol., Vol. 26, No. 6, pp. 965–975, 2000

Cross-section

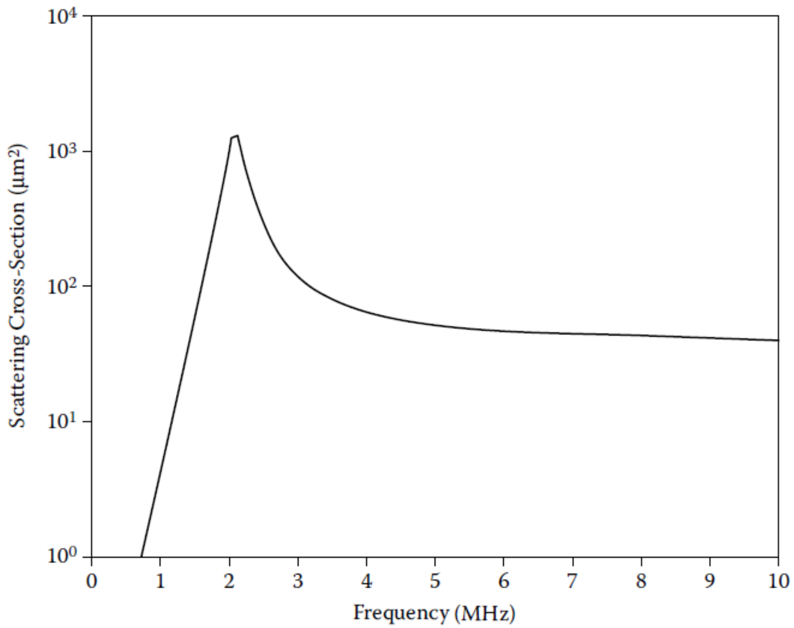


FIGURE 7.2 Calculated scattering cross-section for a free bubble of 1.7-μm radius.

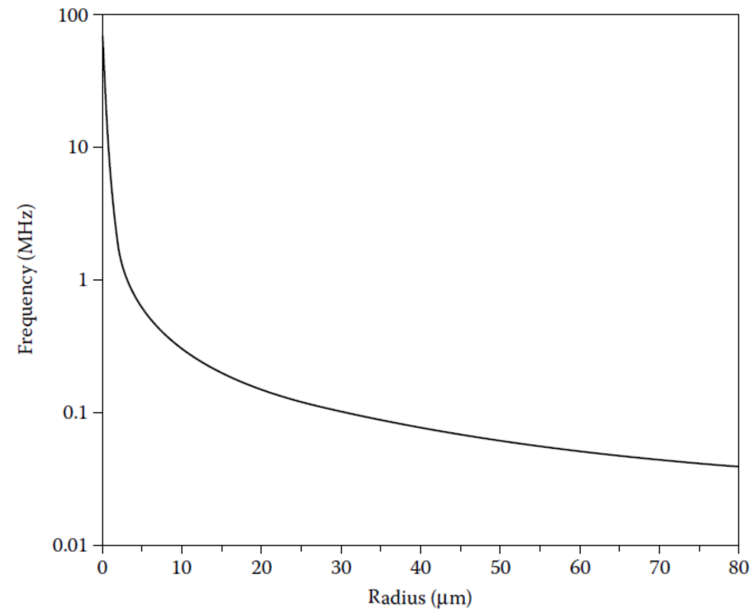
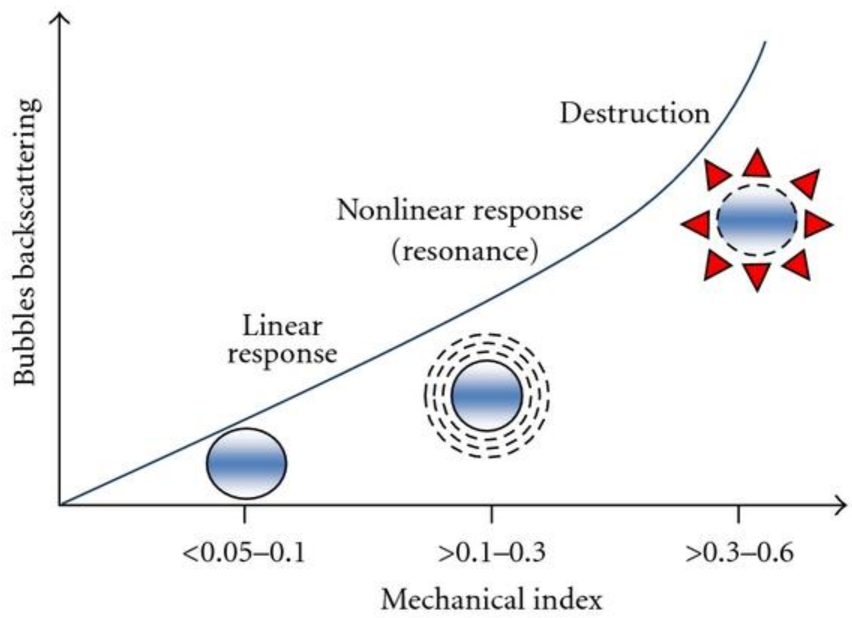


FIGURE 7.1 Calculated resonance frequency vs. radius of a free air bubble.

MB – US interaction

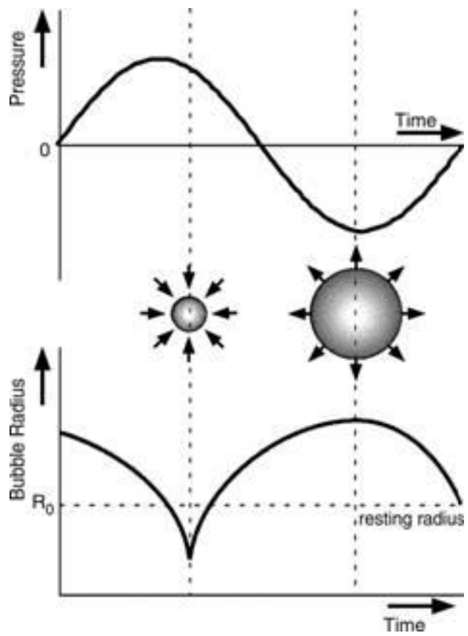
- The response of a gas bubble to a US wave depends on the acoustic pressure amplitude and can be divided into three regimens.



Nonlinear effect

- For higher amplitudes compression generally retards relative to expansion and nonlinearity occurs.
- Bubble size is not linearly related to the applied acoustic pressure.

Because of the finite compressibility of the entrapped air.



Frinking et al. Ultrasound in Med. & Biol., Vol. 26, No. 6, pp. 965–975, 2000

http://bme240.eng.uci.edu/students/08s/chiashel/results.htm

Nonlinear effect

- Bubble vibration contains second and higher multiples of the transmitted frequency.
- The backscattered signal from the bubble not only contains the fundamental (transmitted) frequency, but also harmonic frequencies, most notably at twice the fundamental frequency.

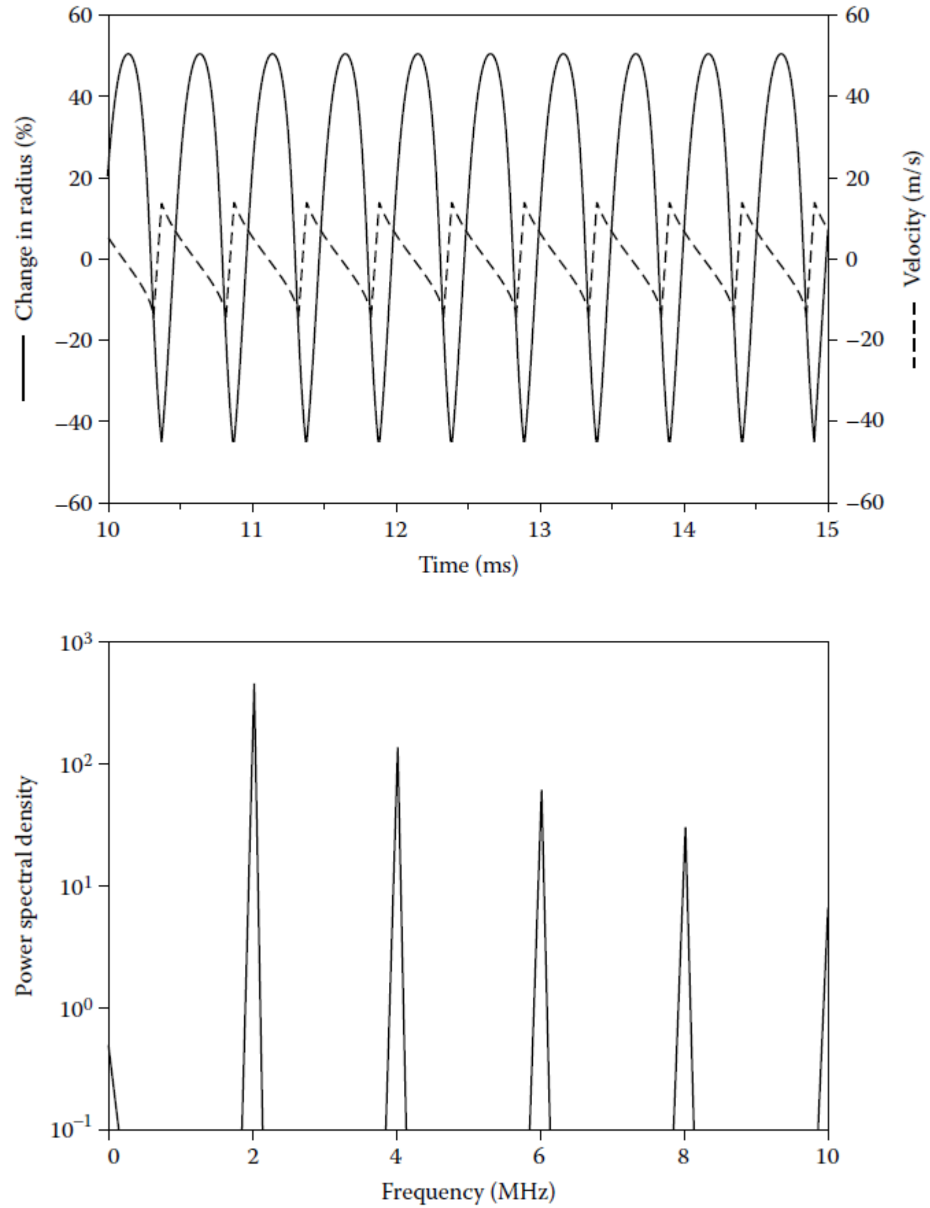
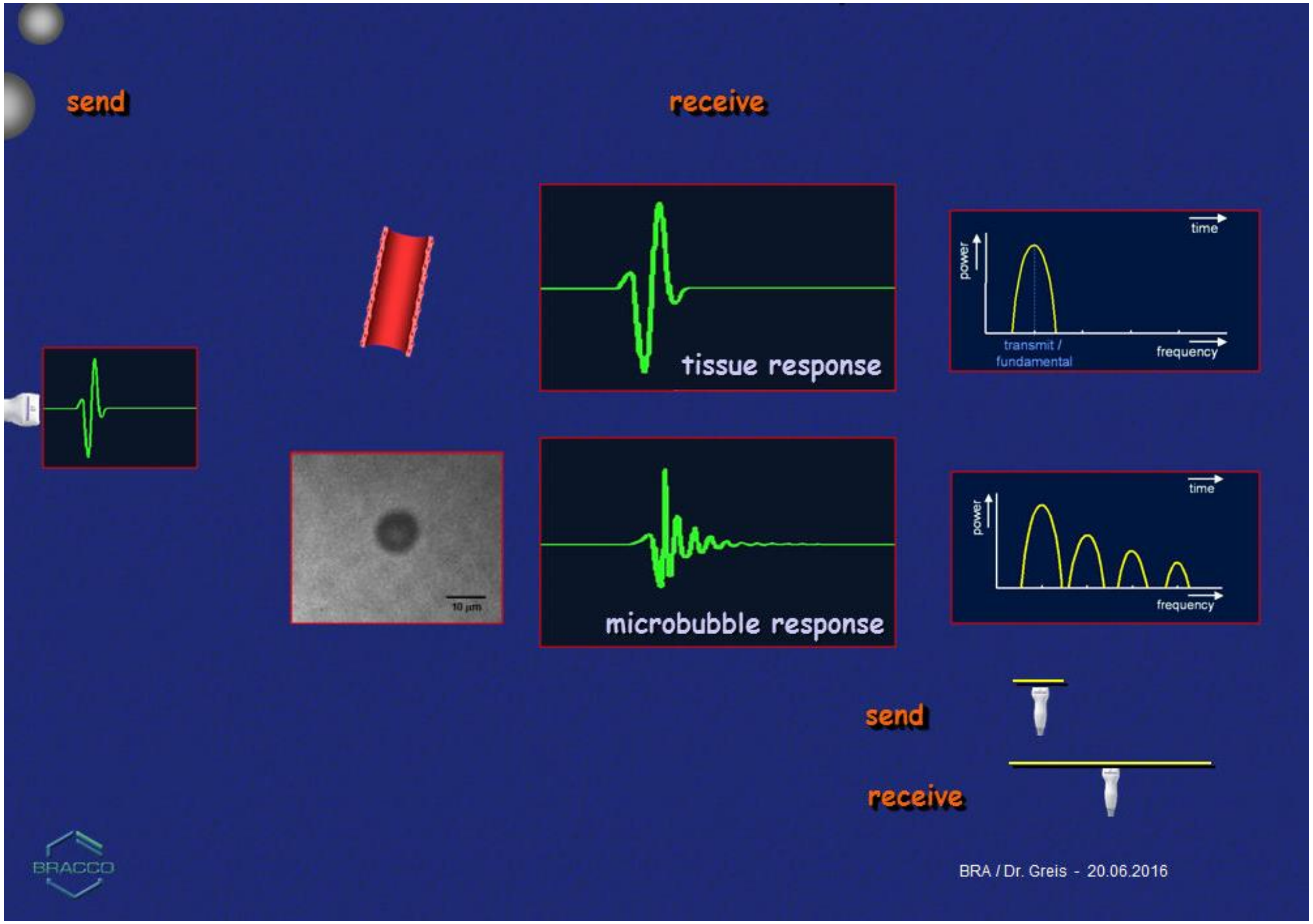


FIGURE 7.5 Response of an air bubble driven at its resonant frequency by an ultrasonic wave of 40 kPa. Top: changes in radius and velocity of bubble wall. Bottom: corresponding power spectra.

Microbubble



http://bme240.eng.uci.edu/students/08s/chiashel/results.htm

http://www.contrastultrasound-modality.com/contrast-ultrasound/what-microbubble/microbubbles-non-linear-oscillation

Harmonic imaging

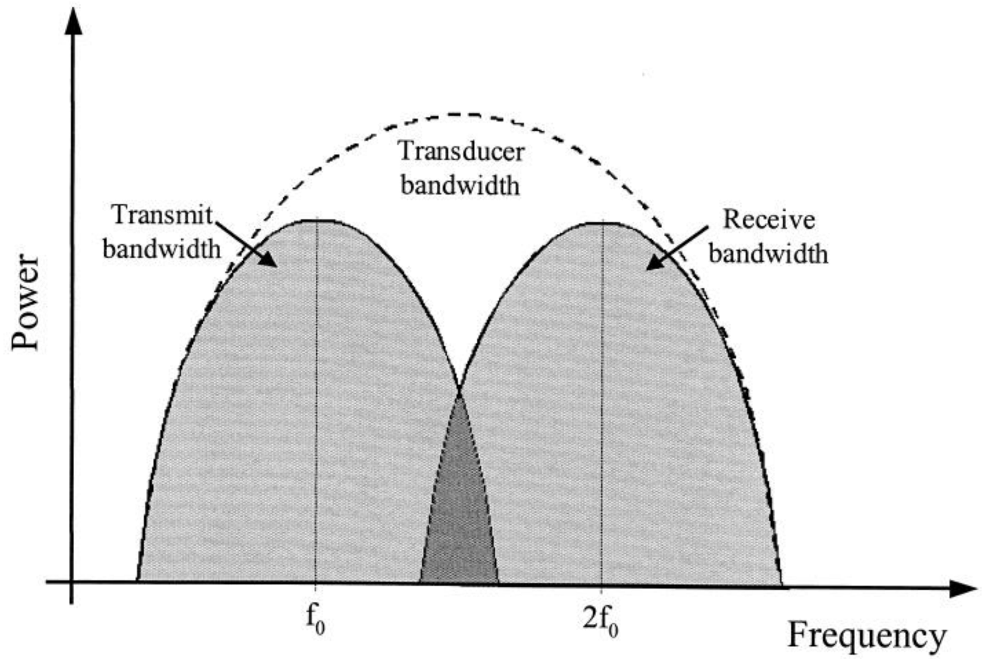
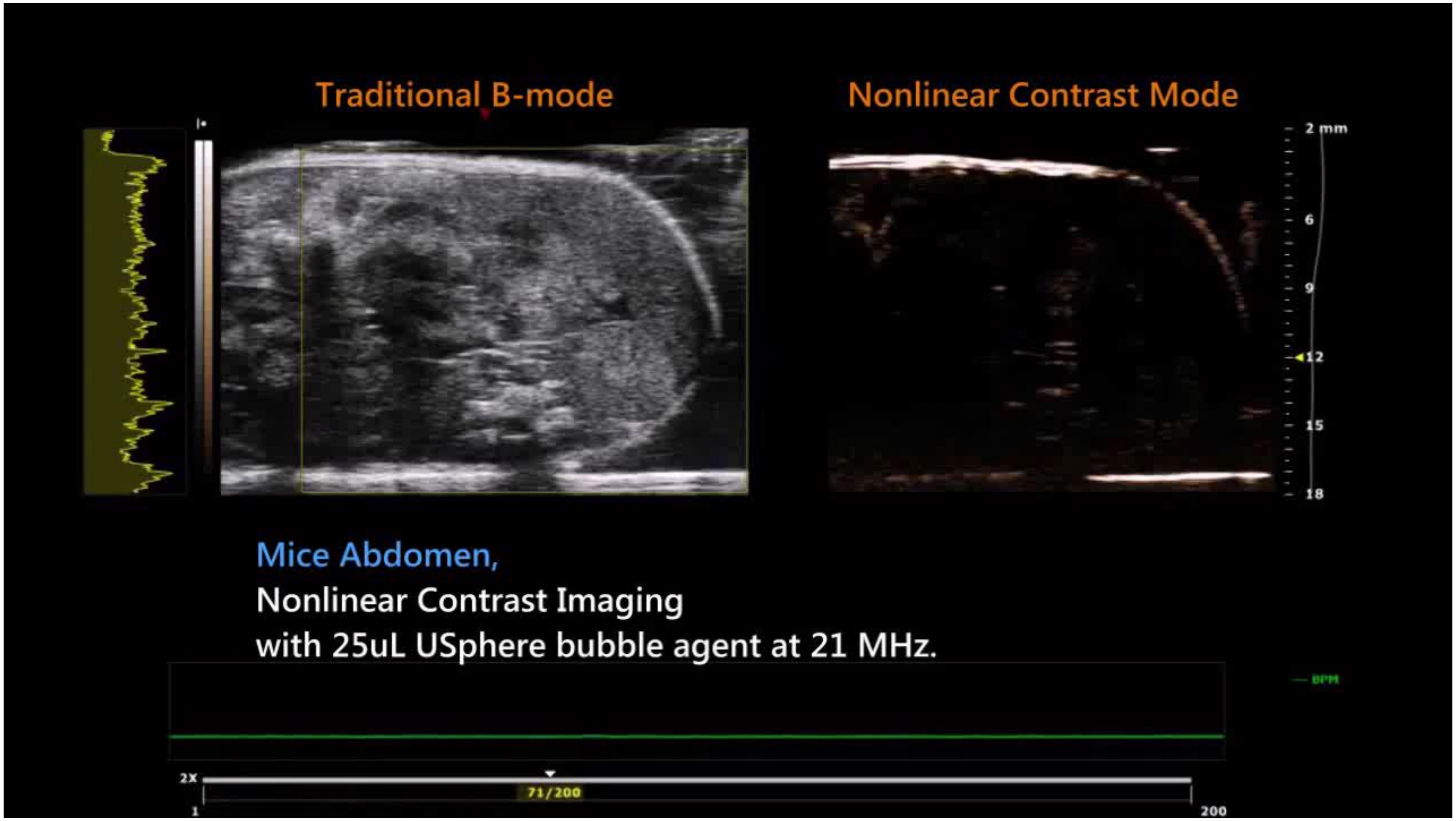


Fig. 2. Overlap between transmit (f_0) and receive ($2f_0$) passbands (dark grey area) results in a residual signal of the fundamental image in the filtered harmonic image.

Harmonic vs B-mode



Example

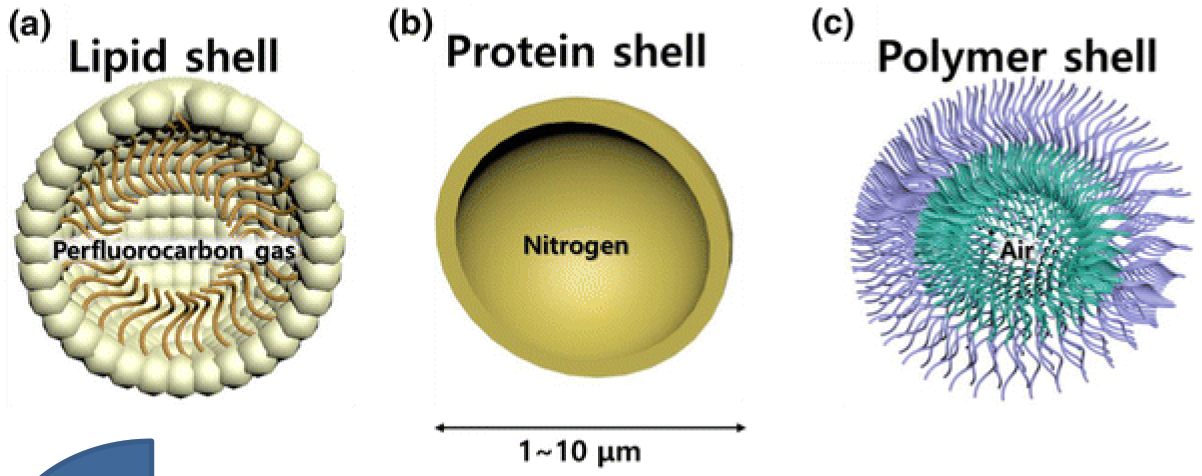
Focal Nodular Hyperplasia with contrast SonoVue

Current status

- The first commercial UCAs became available in the 1980s and included Echovist (1982) and Levovist (1985), which were available in Europe, Japan, and Canada.

- Albunex, the first commercial agent approved by the US - FDA was subsequently released in the USA in 1994. It is an albumin-coated and air-filled microsphere.

Current status



- PFC provides more stable MB because it is not soluble in water.
- Longer lifetime (minutes).

Current status

TABLE 1 | Ultrasound contrast agent that have/had been clinically approved.

Name	First approved for clinical use	Shell material	Gas	Application (examples)	Producer/distributor	Countries
Optison	1998	Cross-linked serum albumin	Octafluoropropane	Left ventricular opafication	GE healthcare, Buckinghamshire, UK	US, Europe
Sonazoid	2007	Phospholipid	Perfluorobutane	Myocardial perfusion, liver imaging	GE healthcare, Buckinghamshire, UK/ Daiichi Saniko, Tokyo, JP	Japan, South Korea
Lumason/SonoVue	2001/2014	Phospholipid	Sulphurhexafluoride	Left ventricular opafication, microvascular enhancement (liver and breast lesion detection)	Bracco diagnostics, Milano, Italy	US, Europe, China
Definity/Luminity	2001/2006	Phospholipid	Octafluoropropane	Echocardiography, liver/kidney imaging (Canada)	Lantheus medical Imaging, North Billerica, MA	North America, Europe (approval filed)
Imagent/Imavist	2002, withdrawn	Phospholipid	Perfluorohexane, Nitrogen	Echocardiography, heart perfusion, tumor/blood flow anomalies	Schering AG, Berlin, DE	US
Echovist	1991, withdrawn	Galactose microparticles	Air	Right heart imaging	Schering AG, Berlin, DE	Germany, UK
Levovist	1995, withdrawn	Galactose microparticles, palmitic acid	Air	Whole heart imaging, doppler imaging	Schering AG, Berlin, DE	Canada, Europe, China, Japan
Albunex	1993, withdrawn	Sonicated serum albumin	air	Transpulmonary imaging	Molecular Biosystems Inc., San Diego, CA, USA	Japan, US

Molecular imaging with microbubbles

Molecular analyses are achieved by coupling specific ligands to the bubbles' shell, which bind to marker molecules in the area of interest.

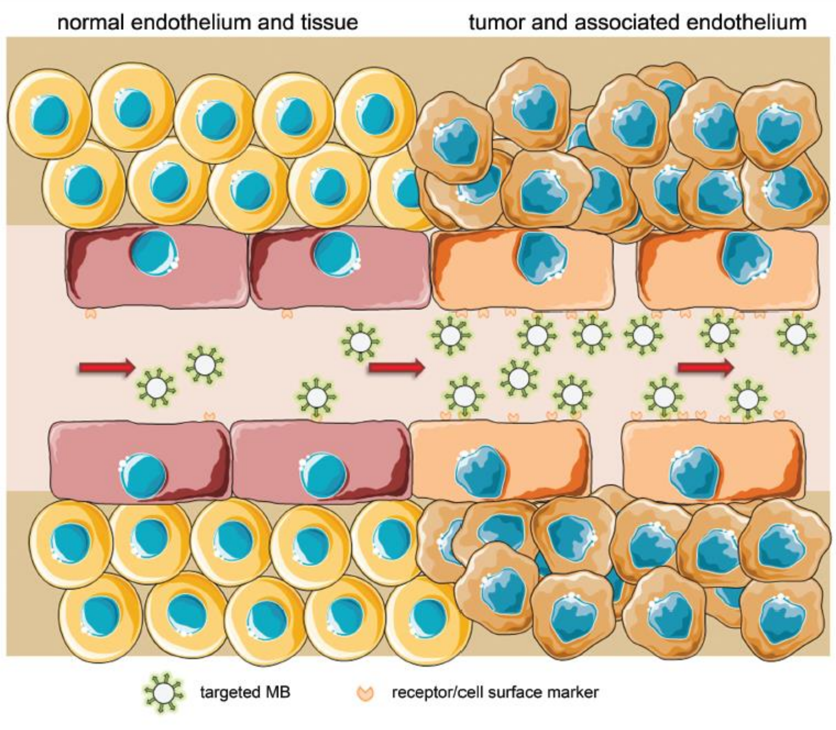
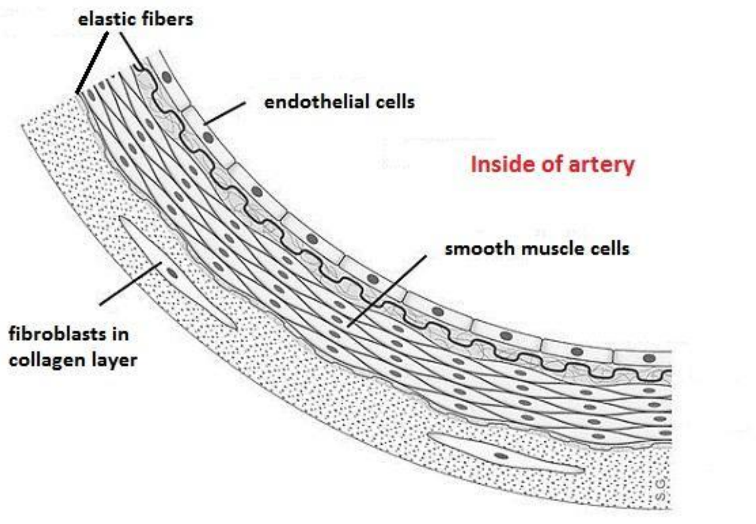


FIGURE 2 | Active targeting by coupling of ligands to MB that bind to structures overexpressed or exclusively expressed on tumor endothelium (schematic illustration, not drawn to scale).

Molecular imaging with microbubbles

- Active targeting requires specific surface modification.
- Since MB are limited to the vascular compartment, their targets need to be expressed on the luminal side of endothelial cells in pathological environments



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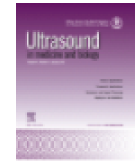
Ultrasound in Medicine & Biology

Volume 41, Issue 1, January 2015, Pages 197-207



Original Contribution

Molecular Ultrasound Imaging Using Contrast Agents Targeting Endoglin, Vascular Endothelial Growth Factor Receptor 2 and Integrin

Ingrid Leguerney *, Jean-Yves Scoazec †, Nicolas Gadot †, Nina Robin ‡, Frédérique Pénault-Llorca ‡, Steeve Victorin *, Nathalie Lassau *

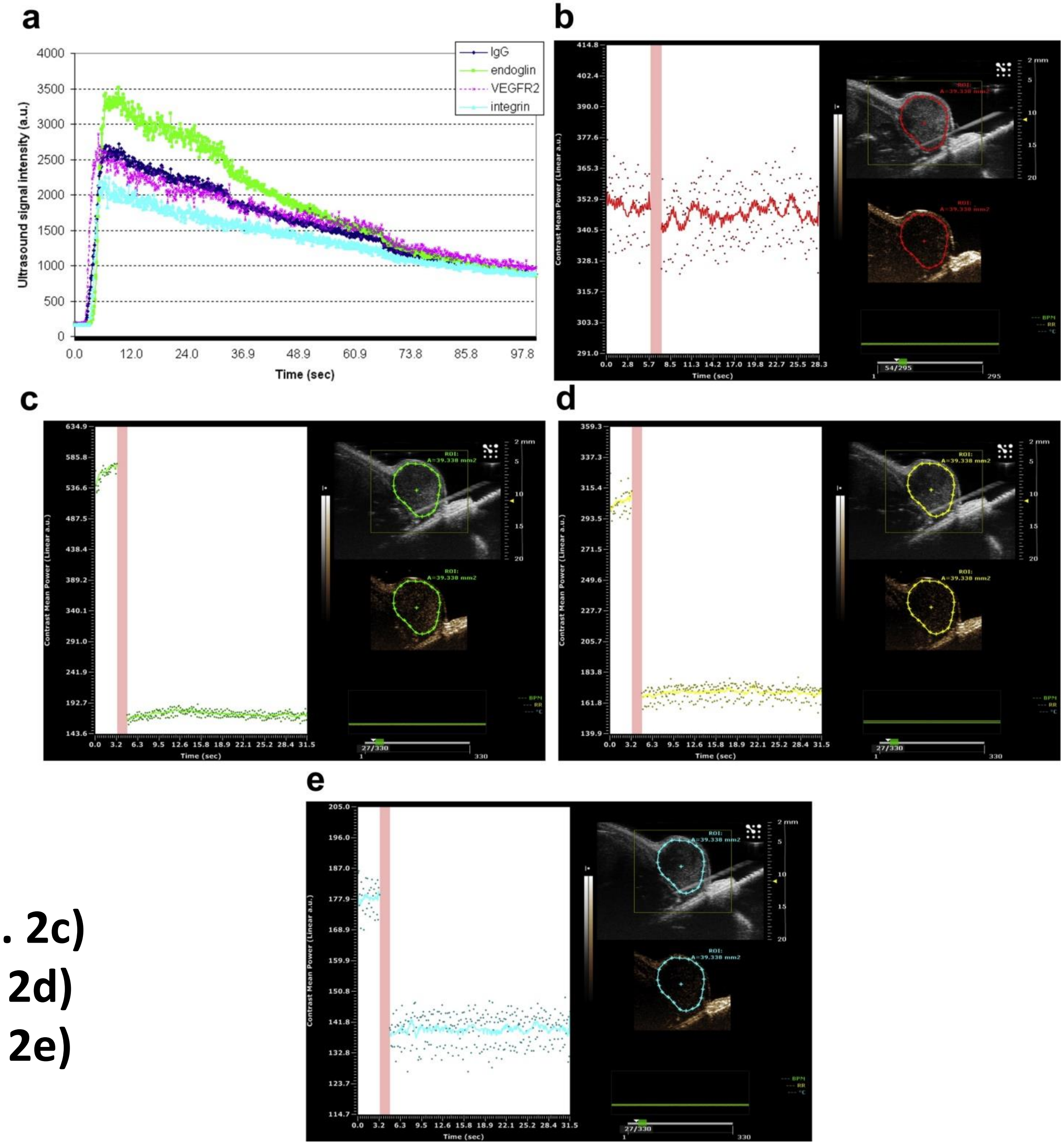
- αvβ3 integrin is highly expressed on activated angiogenic endothelial cells in tumor vessels, resulting in cancers that are more invasive.

- The vascular endothelial growth factor receptor 2 (VEGFR2) is commonly expressed on activated, proliferating endothelial cells, which makes it a target of interest for the detection of tumor angiogenesis.

- Endoglin is a membrane glycoprotein located on cell surfaces and is a component of the transforming growth factor beta receptor complex involved in cell proliferation, differentiation and migration.

Methods

- Thirty-two female immunodeficient nude mice (6–8 wk old).
- Murine B16 F10 melanoma cells (ATCC-CRL-6475) were subcutaneously inoculated into the right flank of the mice.
- Each mouse received successively random boluses of all three different types of functionalized microbubbles targeted.
- Additional random bolus injection of immunoglobulin G (IgG) isotype control targeted microbubbles, without any specific binding.



IgG (Fig. 2b)
Endoglin (Fig. 2c)
VEGFR2 (Fig. 2d)
Integrin (Fig. 2e)

Nanobubble

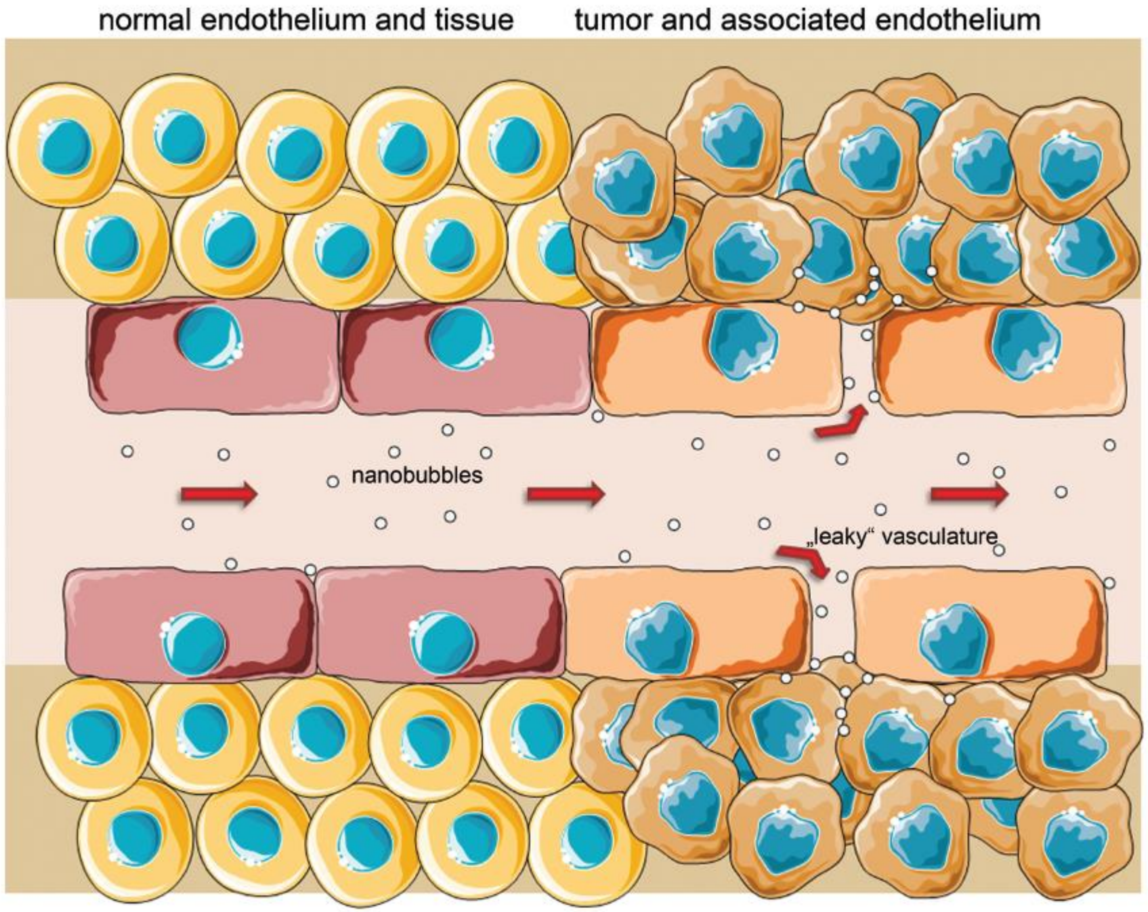


FIGURE 3 | Passive targeting is enabled by 'leaky' vessels with fenestrae up to several 100 nm in tumor-associated endothelium and a poor lymphatic drainage, increasing both likelihood and retention time of nano-sized particles in the interstitium (EPR effect). After extravasation, NB/particles could also actively target specific surface molecules on cancer cells (schematic illustration, not drawn to scale).

International Journal of Nanomedicine

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ORIGINAL RESEARCH

Nanobubbles for enhanced ultrasound imaging of tumors

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[1]Department of Medical Ultrasonic, Third Affiliated Hospital, [2]PCFM Laboratory of the Ministry of Education, School of Chemistry and Chemical Engineering, Sun Yat-Sen University, Guangzhou, People's Republic of China

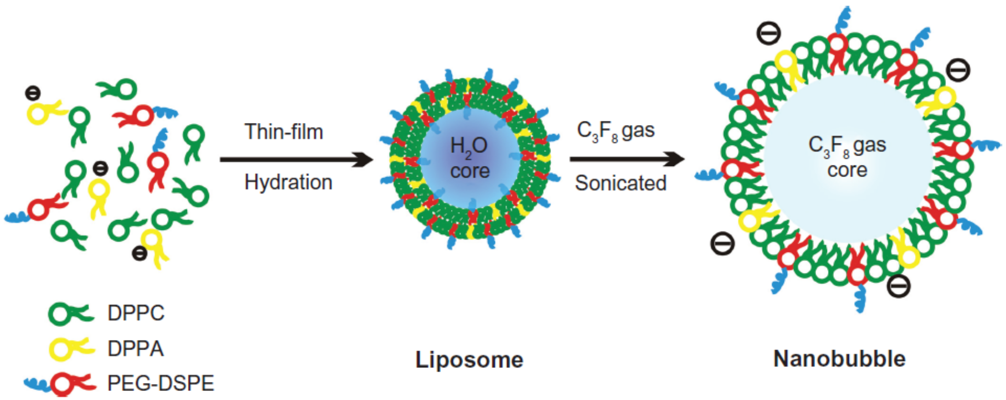


Figure 1 Formation and structural transitions of nanobubbles for ultrasonic imaging and tumor targeting.
Abbreviations: DPPA, 1,2-dipalmitoyl-sn-glycero-3-phosphate; DPPC, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine; PEG-DSPE, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[biotinyl(polyethylene glycol)2000].

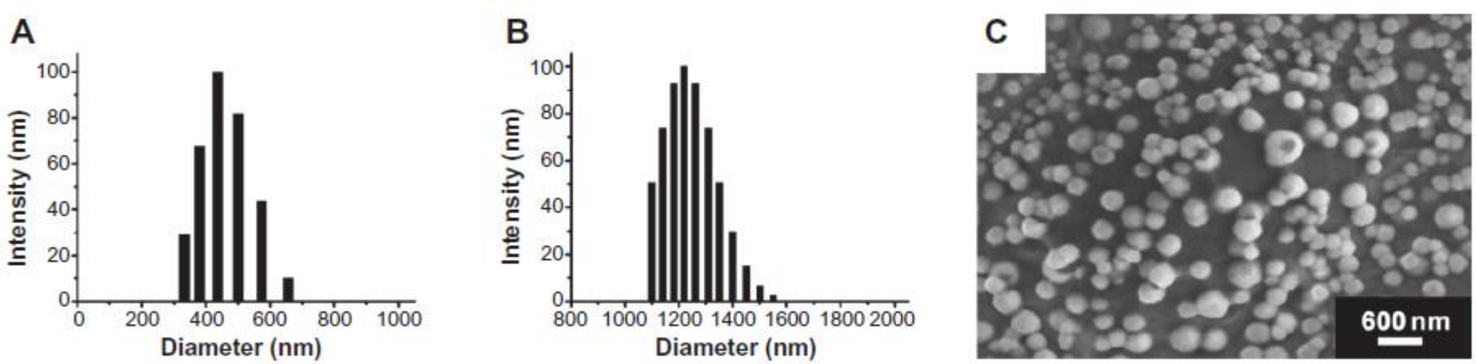
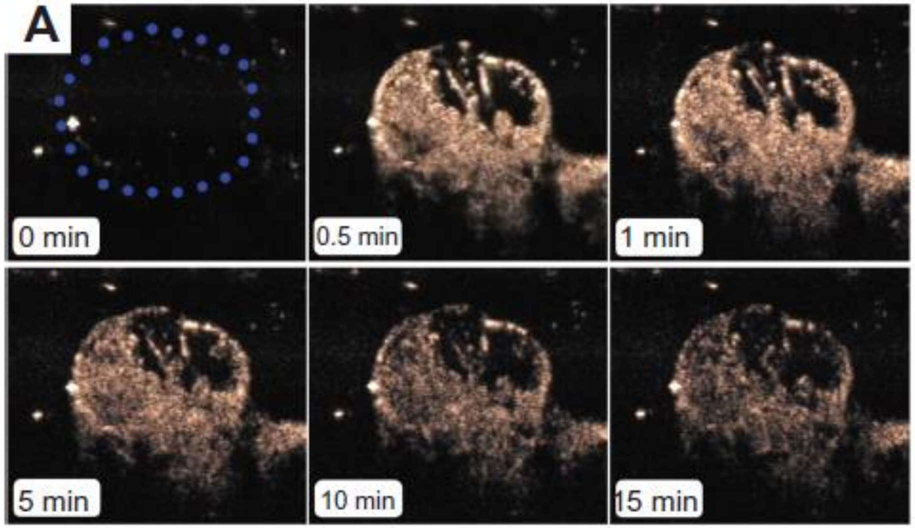
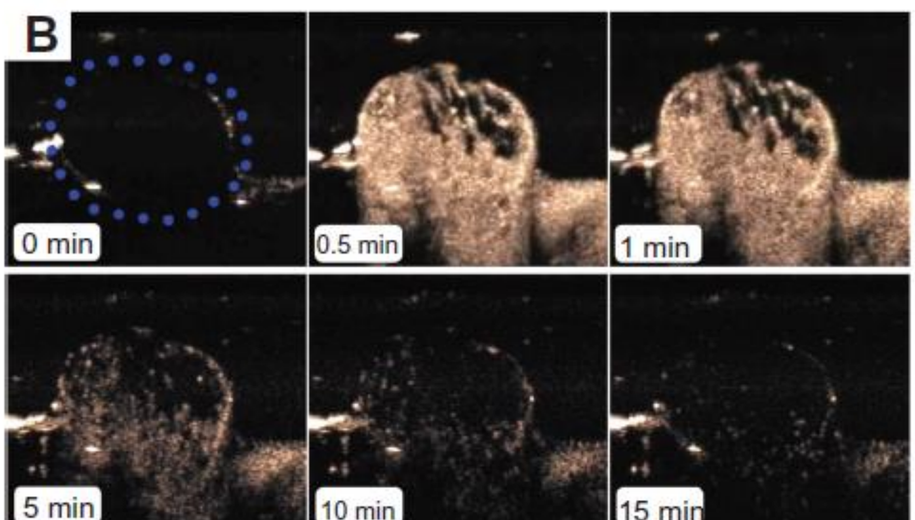


Figure 3 Particle size and morphology of the nanobubbles. The diameter distribution was measured using dynamic light scattering in the nanobubbles (**A**) and microbubbles (**B**). The surface morphology of the nanobubbles was visualized using scanning electron microscopy (**C**).

NB



MB



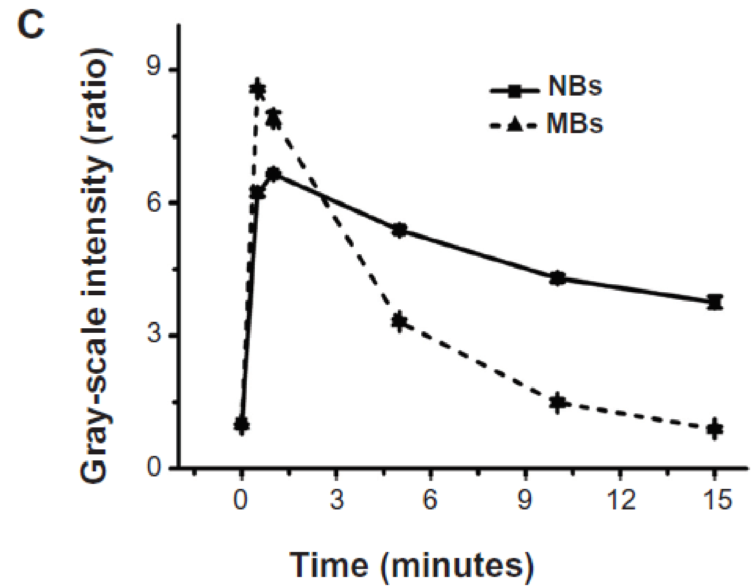
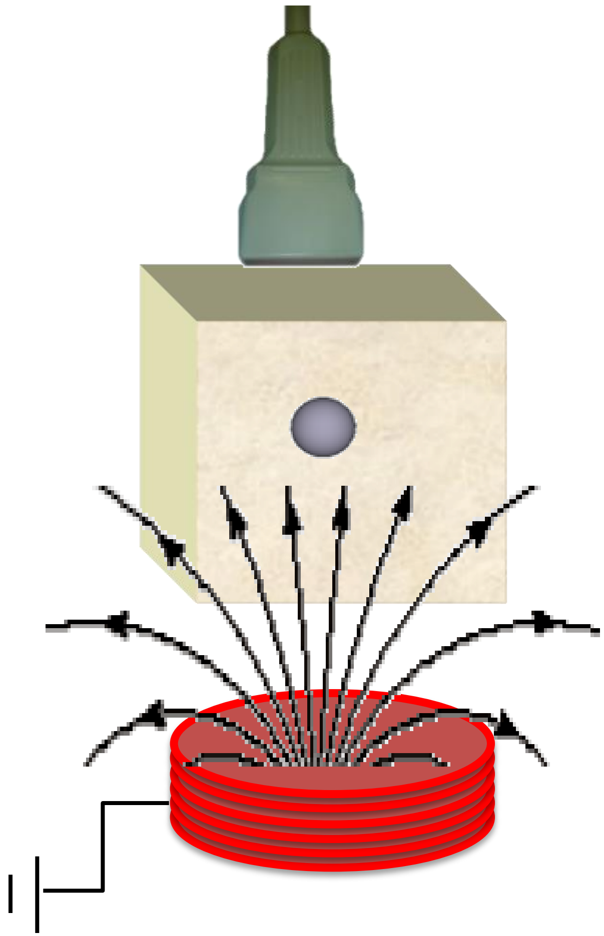
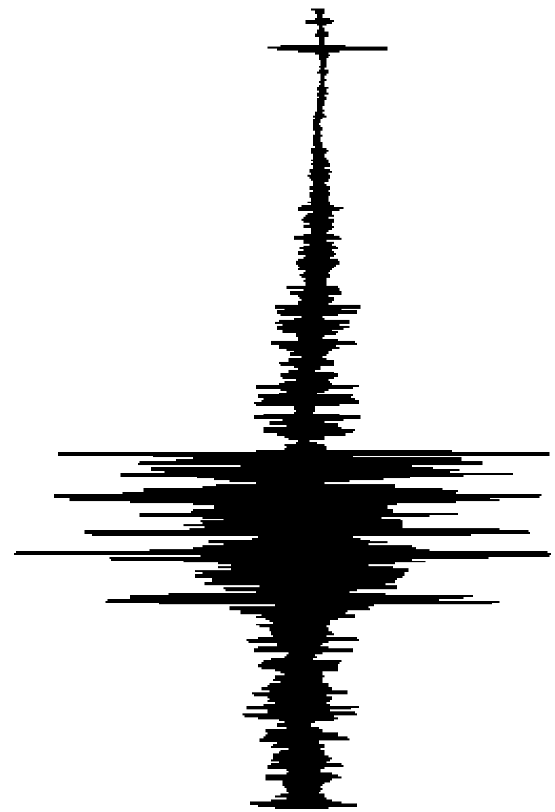


Figure 7 In vivo passive tumor targeting. Representative subcutaneous tumor images before (blue dotted line) and after the injection of nanobubbles (NBs) (**A**) compared with microbubbles (MBs) (**B**) at various time points (0, 0.5, 1, 5, 10, and 15 minutes). The corresponding time–intensity curve of tumor enhancement after injection of the contrast agent (**C**).

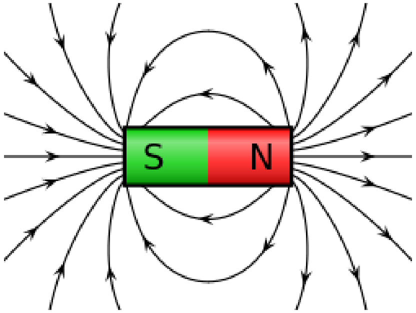
Magneto-motive ultrasound

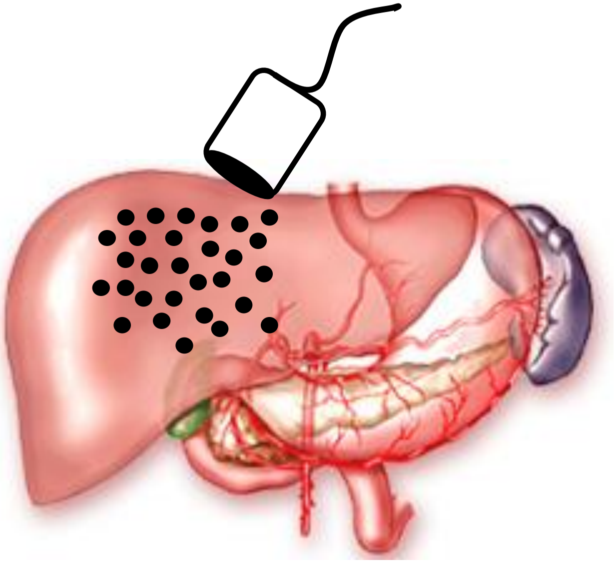




Magneto-motive ultrasound

Ferromagnetic or superparamagnetic particles are displaced by an external magnetic field gradient





Magnetic field

Pulsed

CW

Resulting movement is evaluated using ultrasound

S. A. McAleavey, et. al.*IEEE Transactions on Biomedical Engineering, 2003*

Magneto-motive force

- Any magnetic material submitted to a external magnetic field, with strength **H**, gives rise to a magnetic induction.

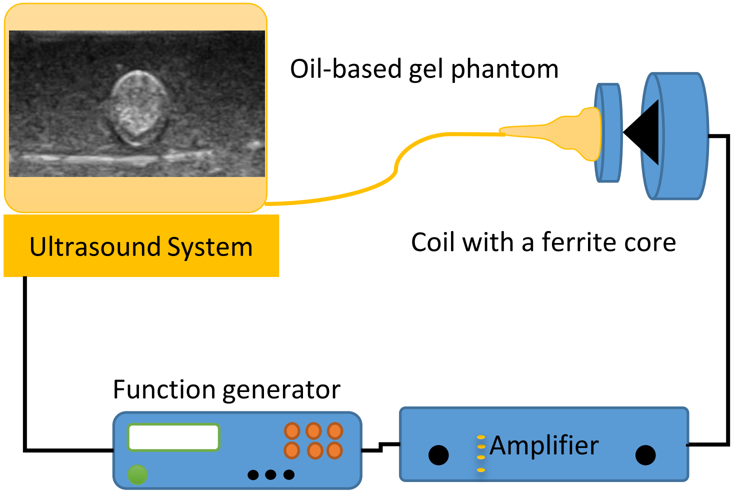
$$\mathbf{B} = \mu_0(\mathbf{H} + \mathbf{M})$$

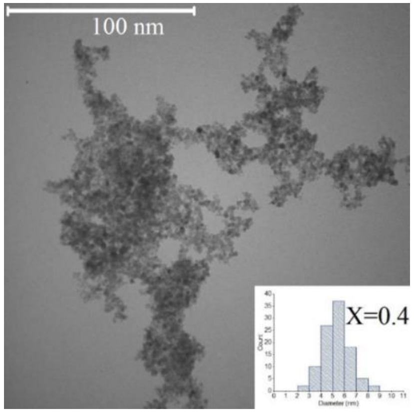
- In the presence of a magnetic field, the particles response to the magnetic translational force.

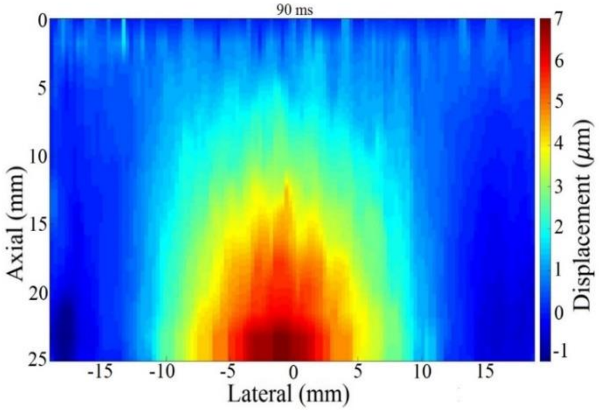
$$\mathbf{F_m} = \frac{V_p \chi_p}{2\mu_0} \boxed{\nabla(|\mathbf{B}|^2)} \qquad F_m = \frac{\chi_p V_p}{2\mu_o}[1 - \cos(4\pi\omega t)]B_z \frac{\partial B_z}{\partial z}$$

Gradient of the magnetic field

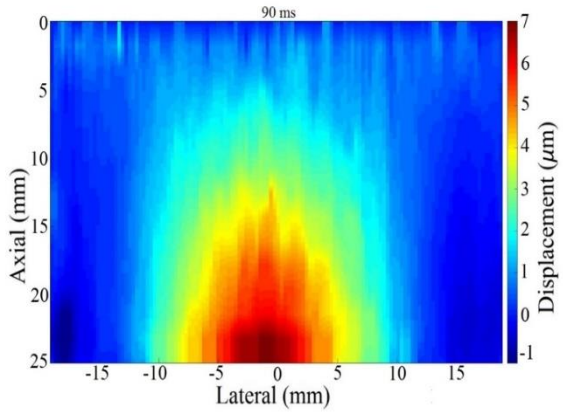
Magneto-motive ultrasound

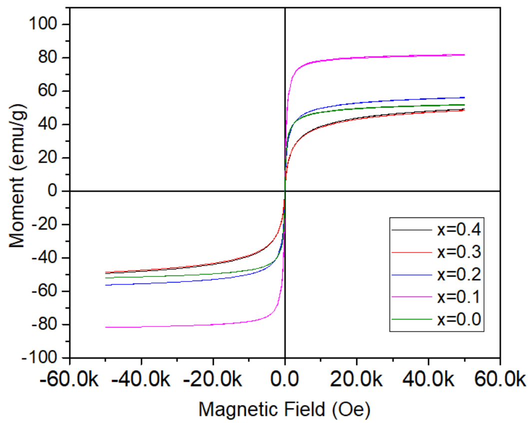


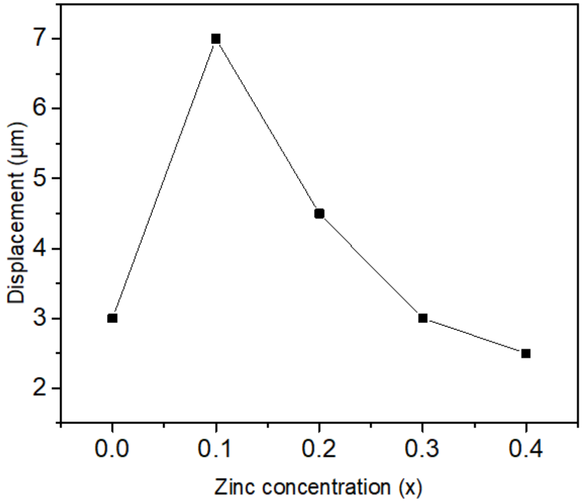


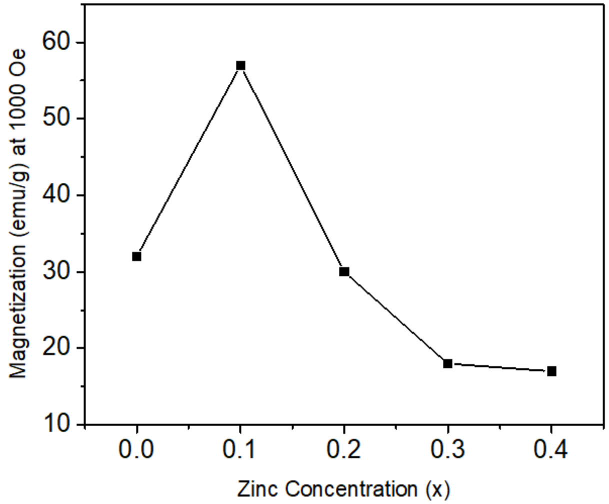


Zn-substituted magnetite ($Zn_xFe_{1-x}Fe_2O_4$)







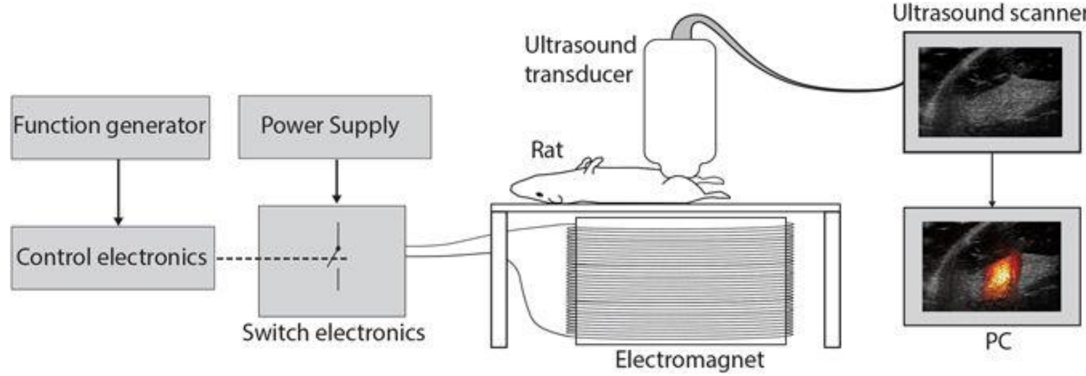


MENU SCIENTIFIC REPORTS

Article | OPEN

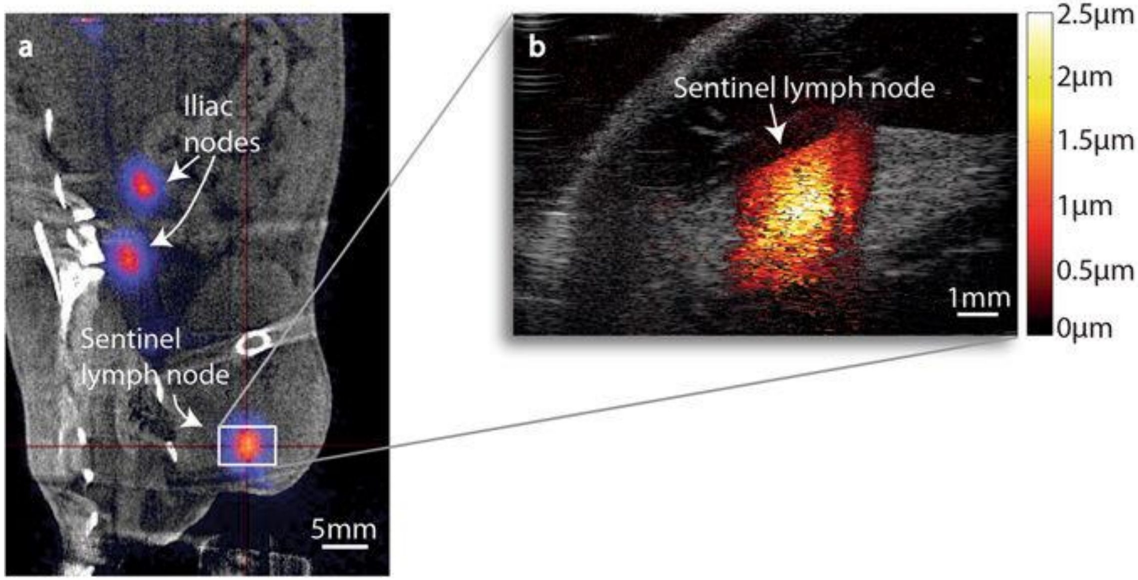
Combined Magnetomotive ultrasound, PET/CT, and MR imaging of ^{68}Ga-labelled superparamagnetic iron oxide nanoparticles in rat sentinel lymph nodes *in vivo*

Maria Evertsson, Pontus Kjellman, Magnus Cinthio, Roger Andersson, Thuy A Tran, Rene in't Zandt, Gustav Grafström, Hanna Toftevall, Sarah Fredriksson, Christian Ingvar, Sven-Erik Strand & Tomas Jansson

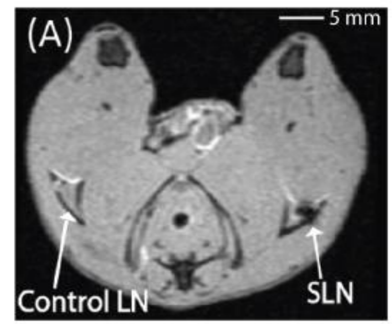


Coronal PET/CT

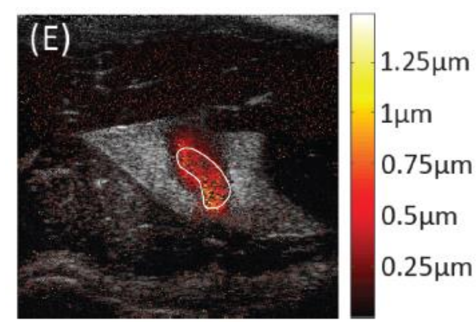
MMUS



MRI



MMUS



Motivation

Ingestion of a test food marked with a magnetic material

⬇

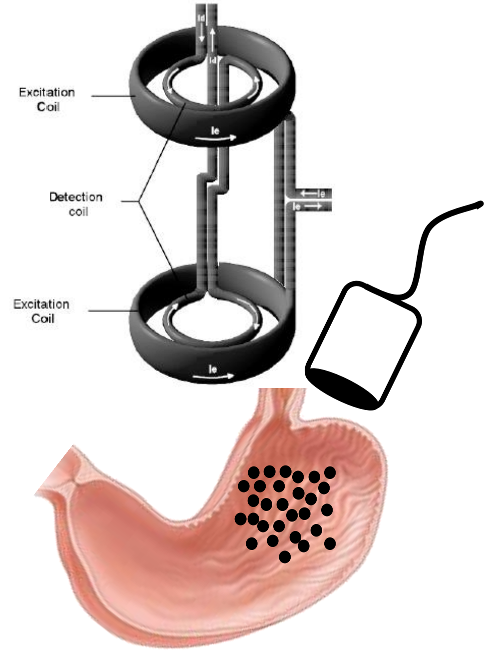
Study gastrointestinal motility

Biosusceptometry → lower spatial resolution

Magneto motive ultrasound

⬇

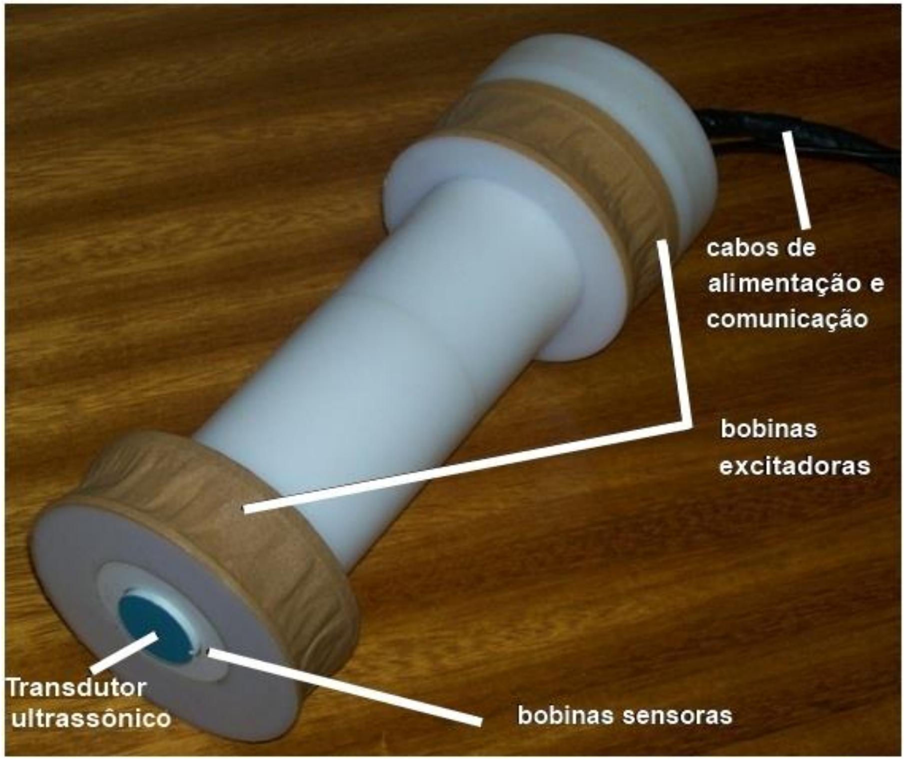
Spatial information during biosusceptometry



A. C. Bruno, T. Z. Pavan, O. Baffa and A. A. O. Carneiro, IEEETUFFC, 2013

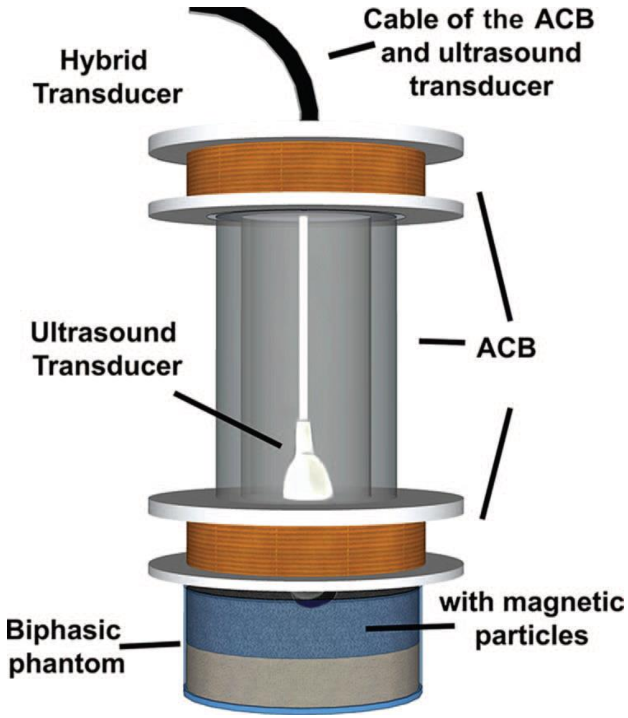
Localização de partículas

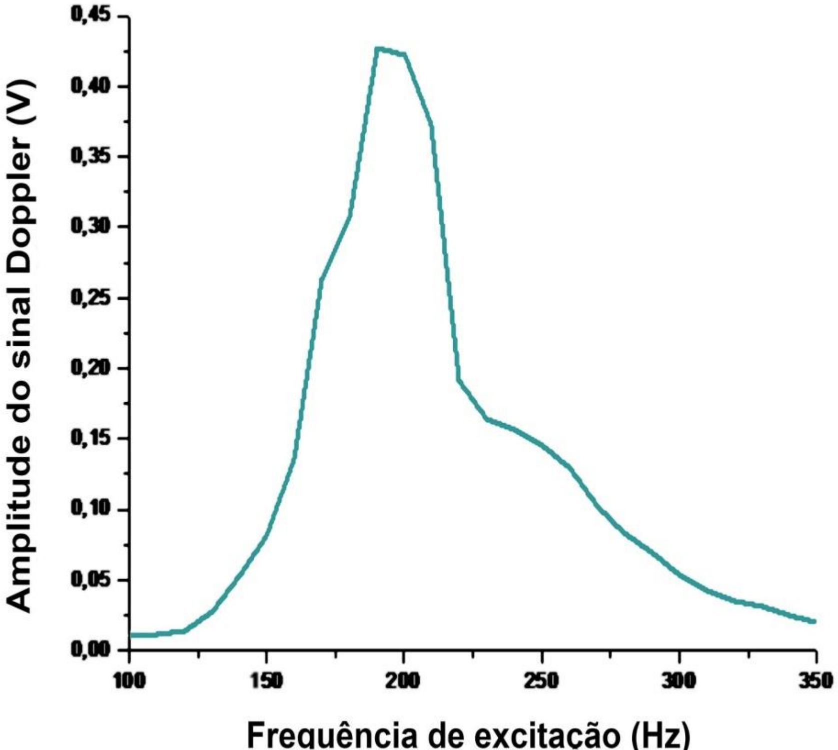
- Transdutor Híbrido
  - Gastrointestinais
    - Motilidade
    - Elastografia

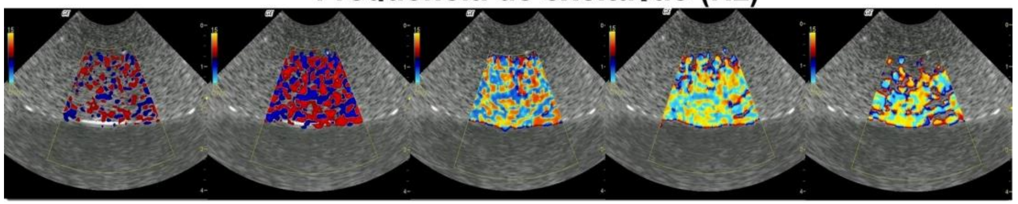


A. Colello Bruno, T. Z. Pavan, O. Baffa, and A. A. Oliveira Carneiro, “A hybrid transducer to magnetically and ultrasonically evaluate magnetic fluids,” *IEEE Trans. Ultrason. Ferroelectr. Freq. Control*, vol. 60, no. 9, pp. 2004–2012, 2013.

Localização de partículas *in vitro*



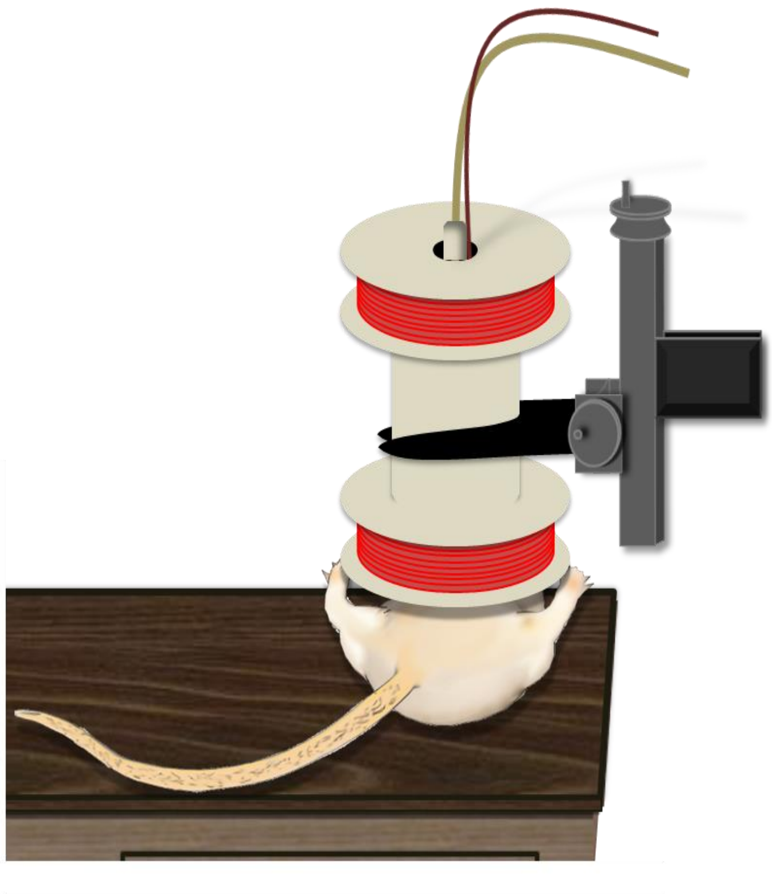




100 Hz 150 Hz 200 Hz 250 Hz 300 Hz

A. C. Bruno, T. Z. Pavan, O. Baffa and A. A. O. Carneiro, IEEETUFFC, 2013

In vivo viability study



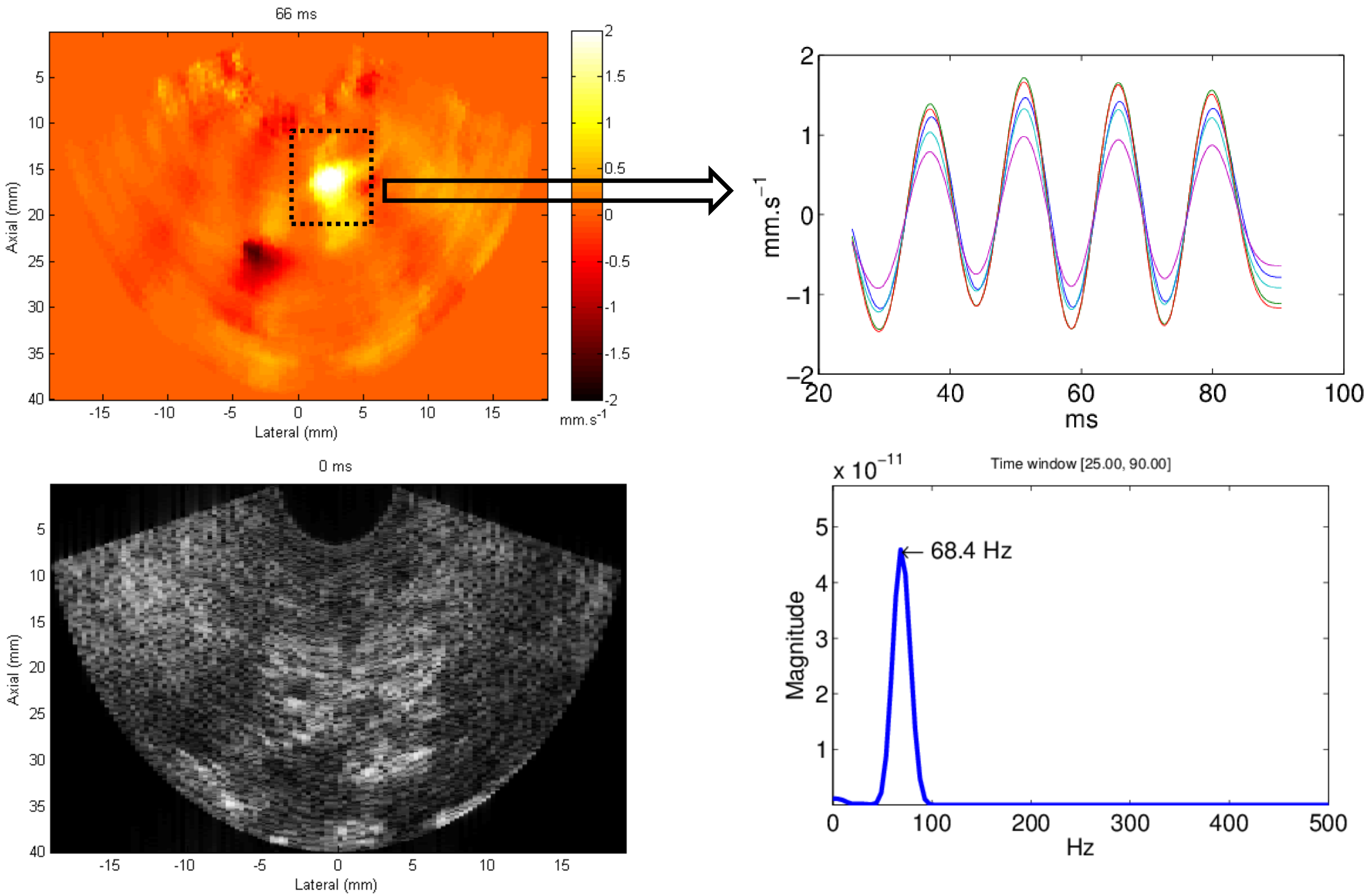
Four male Wistar rats (weighting 300–350 g)

A gavage needle was used to deliver the meal directly into the stomach

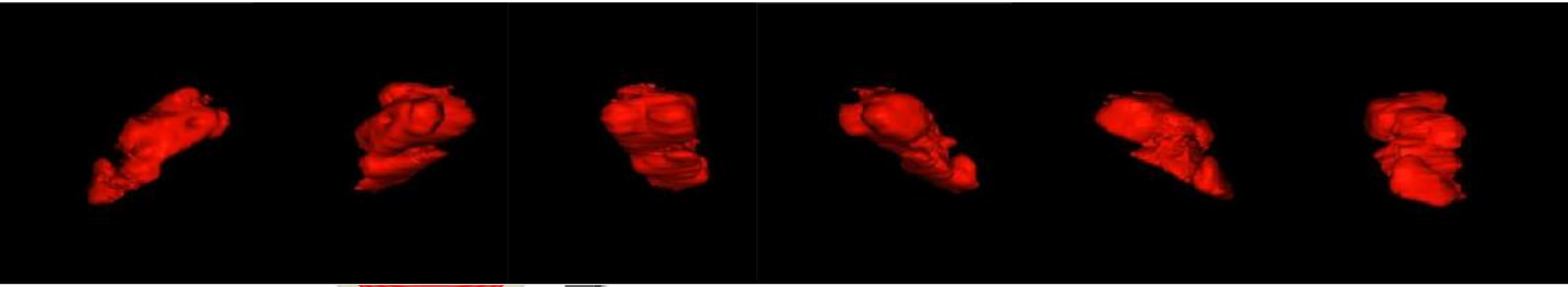
Meal →ferrite particles (diameter between 37 and 70 μm) mixed with yogurt.

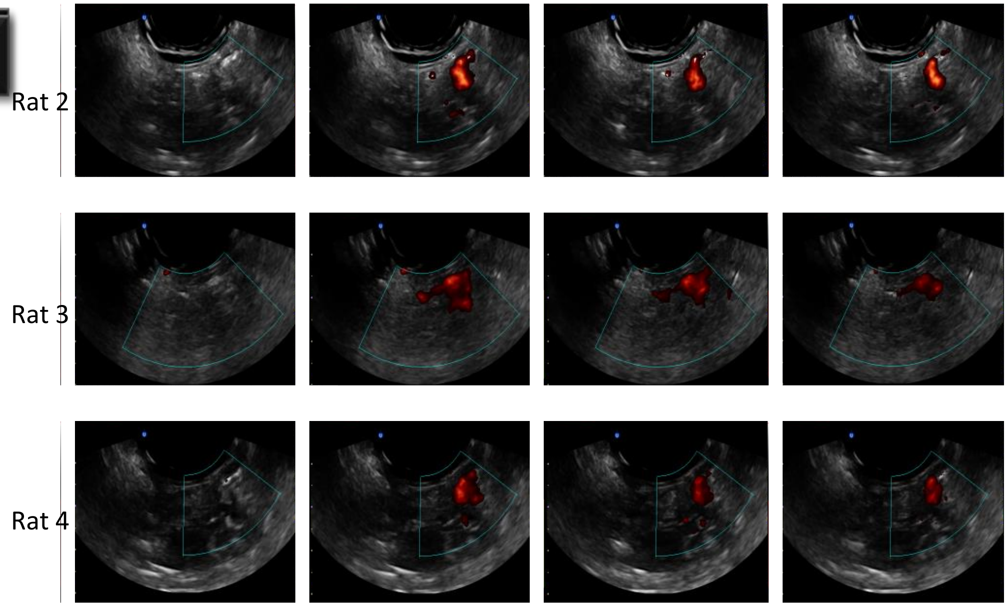
Bruno et al. IEEEIUS, 2014

Magneto Motive Response



Localização de partículas *in vivo* (Motilidade)





Therapeutics

Drug-delivery

- Bubbles can also be loaded with or attached to drugs, peptides or genes and can be destroyed by US pulses to locally release the entrapped agent.

Drug-delivery

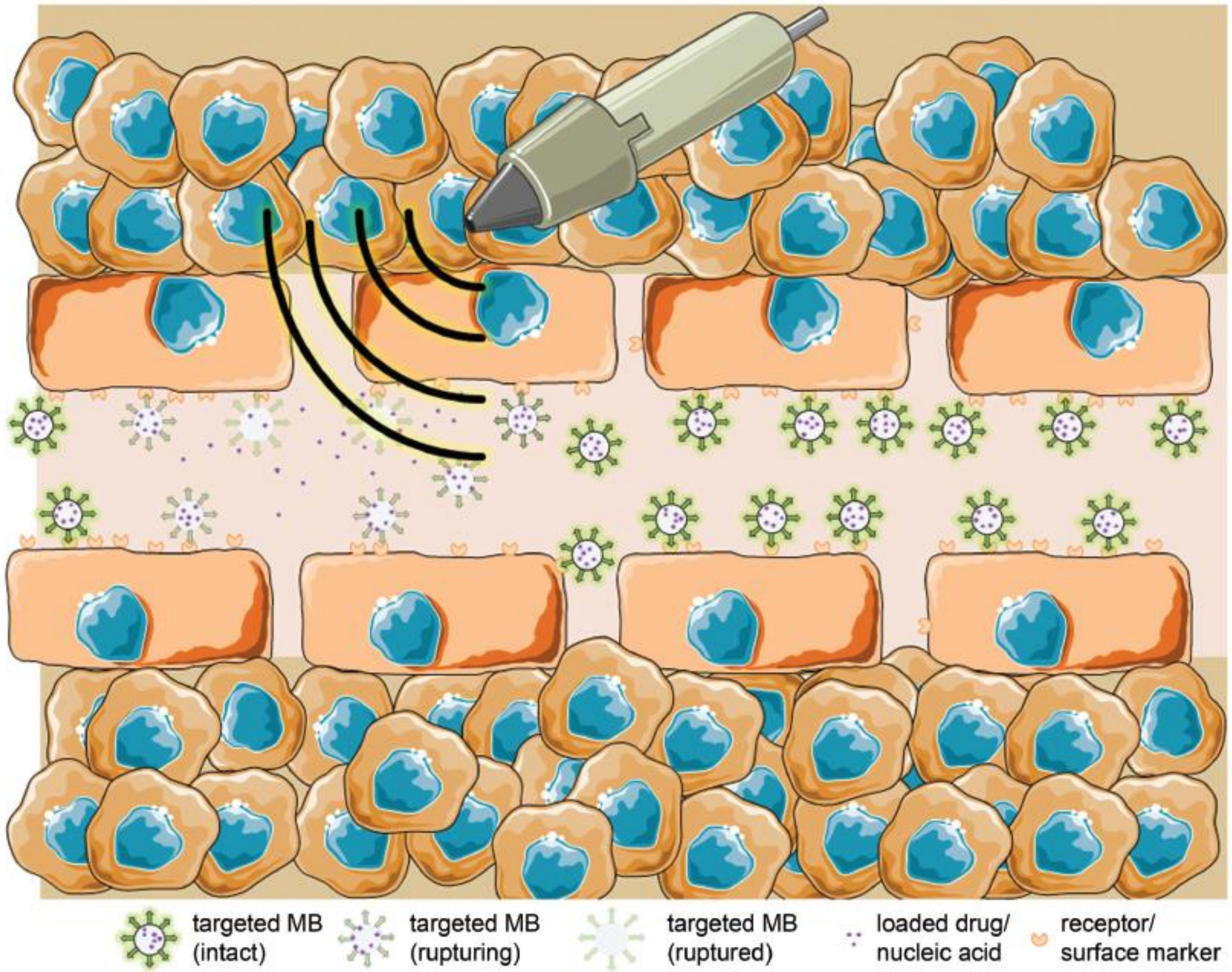


FIGURE 4 | Targeted MBs with entrapped drug/nucleic acids rupture under the US-induced acoustic pressure and release their loading specifically at the side of a tumor (schematic illustration, not drawn to scale).

IVYSPRING INTERNATIONAL PUBLISHER

Theranostics

2012; 2(12):1174-1184. doi: 10.7150/thno.4410

Research Paper

Theranostic Oxygen Delivery Using Ultrasound and Microbubbles

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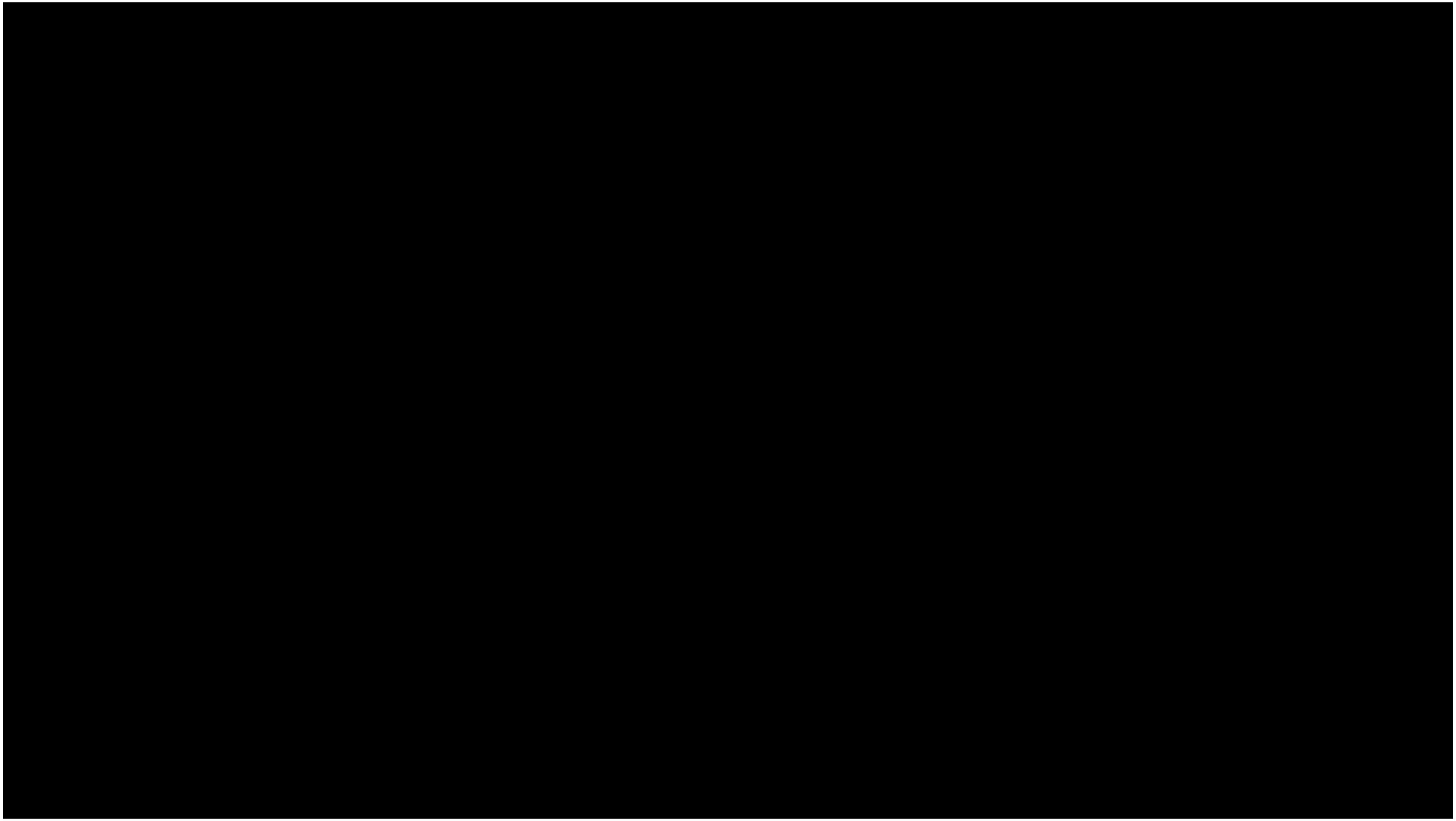
Abstract

Means to overcome tumor hypoxia have been the subject of clinical investigations since the 1960's; however these studies have yet to find a treatment which is widely accepted. It has been known for nearly a century that hypoxic cells are more resistant to radiotherapy than aerobic cells, and tumor hypoxia is a major factor leading to the resistance of tumors to radiation treatment as well as several cytotoxic agents. In this manuscript, the application of ultrasound combined with oxygen-carrier microbubbles is demonstrated as a method to locally increase dissolved oxygen. Microbubbles can also be imaged by ultrasound, thus providing the opportunity for image-guided oxygen delivery. Simulations of gas diffusion and microbubble gas exchange show that small amounts (down to 5 vol%) of a low-solubility osmotic gas can substantially increase microbubble persistence and therefore production rates and stability of oxygen-carrier microbubbles. Simulations also indicate that the lipid shell can be engineered with long-chain lipids to increase oxygen payload during in vivo transit. Experimental results demonstrate that the application of ultrasound to destroy the microbubbles significantly enhances the local oxygen release. We propose this technology as an application for ultrasound image-guided release of oxygen directly to hypoxic tissue, such as tumor sites to enhance radiotherapy.

Key words: Hypoxia, Tumor, Radiotherapy, Oxidation, Oxygenation

Blood Brain Barrier



Sonotrombolysis

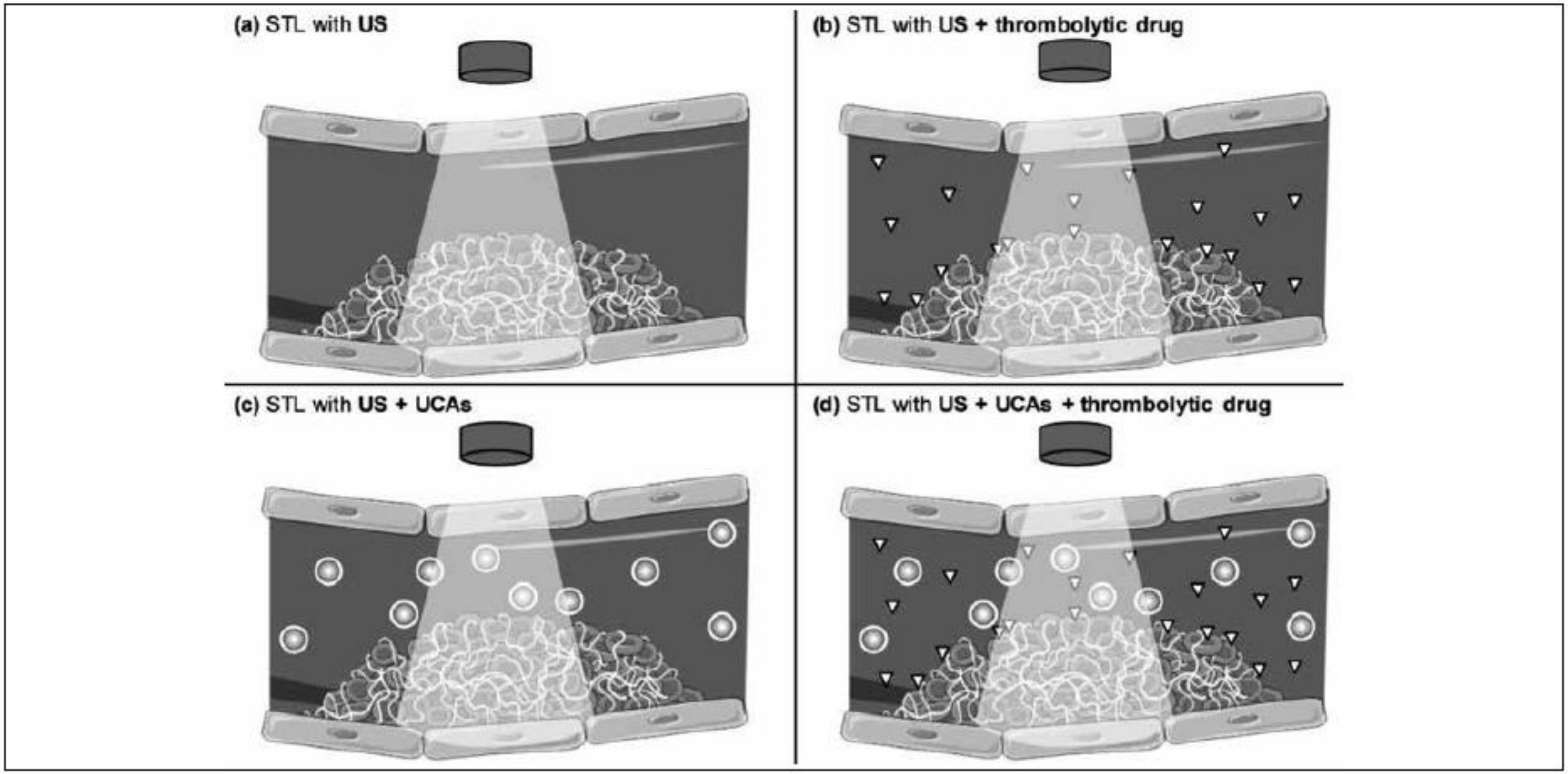


Figure 1 - Schematic representation of the different types of sonothrombolysis (STL): (a) US alone, (b) US + thrombolytic drug, (c) US + UCAs, and (d) US + UCAs + thrombolytic drug (this figure was produced using Servier Medical Art).

Petit et al. J. DRUG DEL. SCI. TECH., 22 (5) 381-392 2012

Microbubble

- The volume pulsation of the bubble is frequency-dependent and shows a clear maximum at a specific frequency, which is referred to as the resonance frequency, and is inversely related to the bubble size.

- The resonance phenomenon is an important effect because a resonating bubble behaves as a source of sound, rather than as a passive reflector and, therefore, yields an enhancement of the backscatter signal compared to the off-resonance behavior of the bubble.

Frinking et al. Ultrasound in Med. & Biol., Vol. 26, No. 6, pp. 965–975, 2000