Ultrasound Microbubble Applications in Radiotherapy

John Eisenbrey, PhD

Department of Radiology, Thomas Jefferson University, Philadelphia, PA 19107, USA

Disclosures

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- Contrast agent support for Lantheus Medical Imaging.
- Research funding from GlaxoSmithKline
- Equipment loan from Toshiba
- Equipment loan from Siemens

Ultrasound Contrast Agents

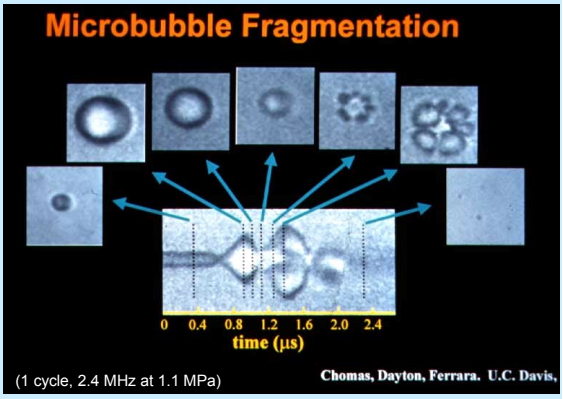
- Gas filled 1 to 8 μm bubbles
- Injected intravenously and transpulmonary
- Generally contain higher molecular weight gasses
- Bubbles are encapsulated:
  - Albumin or polymer shell
  - Lipid or surfactant coated

  for longevity
- Signals mainly from vessels 20 - 40 μm
- FDA approved for cardiac and liver imaging




Contrast-Enhanced Ultrasound for Treatment Response of HCC

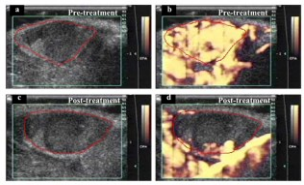
HCV Male with 9.2cm HCC 2 weeks post lipiodol chemoembolization

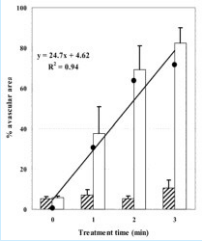


Microbubble applications in radiotherapy

- Microbubble cavitation for sensitizing HCC tumors to radiotherapy
- Microbubble-assisted oxygen delivery for overcoming tumor hypoxia

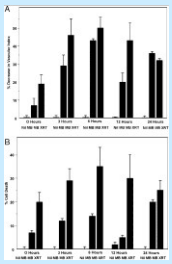
Inertial microbubble cavitation results in reduced tumor vascularity



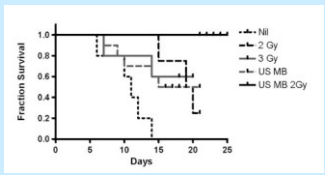


Wood AKW, Ansaloni S, Ziemer LS, Lee WM-F, Feldman MD, Sehgal CM. THE ANTIVASCULAR ACTION OF PHYSIOTHERAPY ULTRASOUND ON MURINE TUMORS. *Ultrasound in medicine & biology*. 2005;31(10):1403-1410.

Inertial cavitation initiated vascular disruption leads to radiosensitivity



* Czarnota GJ, Karshafian R, Burns PN, Wong S, Al Mahrouki A, Lee JW, Caissie A, Tran W, Kim C, Furukawa M, Wong E, Giles A. Tumor radiation response enhancement by acoustical stimulation of the vasculature. Proc Natl Acad Sci U S A. 2012 Jul 24;109(30):E2033-41



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"experimental results supported the role of ceramide signaling as a key element in cell death initiation with treatments using US/MB+XRT to target endothelial cells."
-Al-Mahrouki AA, Wong E, Czarnota GJ. Ultrasound-stimulated microbubble enhancement of radiation treatments: endothelial cell function and mechanism. Oncoscience. 2015;2(12):944-57

Hypothesis:

- Noninvasive microbubble cavitation can be used to sensitize hepatocellular carcinoma prior to radiotherapy, without compromising liver function.

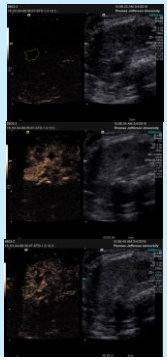
HCC Animal Studies

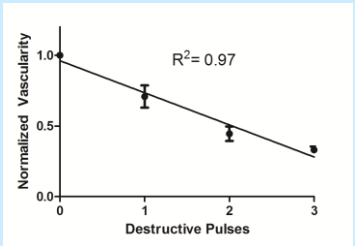
- Human HCC cell line implanted into liver of aythymic nude rats (n=18).
- Tumor growth monitored with 2D ultrasound until maximum tumor diameter > 5 mm.
- Rats randomized into groups receiving: microbubble cavitation alone (0.1ml Optison, GE Healthcare); radiation alone (5 Gy single fraction); or microbubble cavitation 3-4 hours prior to radiotherapy.
- Ultrasound was performed using an S3000 scanner with 9L4 probe and flash-replenishment and a nonlinear imaging package (MI = 1.13 at 4.0 MHz, transmitting 2.3 µs pulses at a pulse repetition frequency of 100 Hz; Siemens Healthineers, Mountain View, CA).
- Imaging plane was maintained at tumor midline for 4 destructive pulses before being swept through the tumor for the remainder of contrast enhancement (approximately 10 pulses).

HCC Animal Studies

- Immediately following UTMD, tumors were marked via placement of a 25G metal tip under ultrasound guidance.
- Approximately 3 hours following UTMD, animals in radiation groups received 5 Gy delivery using 3D conformal radiotherapy guided with cone beam CT guidance (Xstrahl, Camberley, United Kingdom).
- Changes in weight and animal distress were monitored and compared between groups.
- Tail vein blood was collected prior to tumor inoculation, immediately prior to therapy, and 2 days and 1 week post treatment to assess liver function.
- Tumor volumes were measured 2 times per week using 3D imaging on a Vevo2100 with 32MHz probe (VisualSonics, Toronto, Canada).
- Animal survival was monitored and compared between groups (animals sacrificed at 20% weight loss or tumors >1.5 cm based on IACUC conditions).

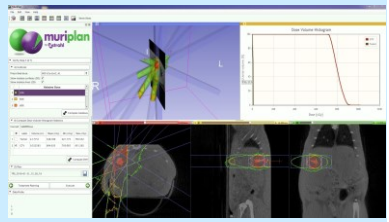
Microbubble Cavitation



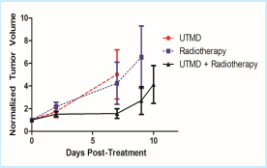


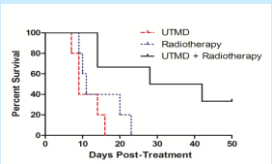
HCC Tumor Marking

Radiation Therapy- Small Animal Radiation Research Platform



HCC Tumoral Response



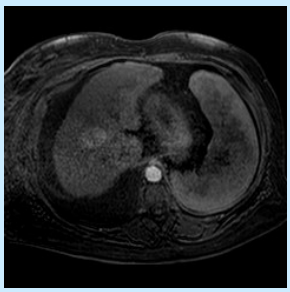


- A 49% reduction in tumor growth rate was observed when radiotherapy was combined with UTMD (top left figure).
- A 56% improvement in survival time was observed when radiotherapy was combined with UTMD (p=0.034).
- No differences in liver function (ALT and bilirubin) were observed between groups receiving radiotherapy (p>0.31).

HCC Cavitation Conclusions:

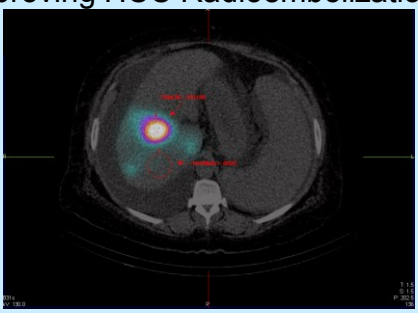
- Microbubble-based antivascular therapy appears safe prior to radiotherapy of HCC, with no detected changes in liver function tests.
- Ultrasound triggered microbubble destruction prior to external beam radiation therapy appears to sensitize tumors to treatment, improving tumoral response and survival in an animal model.
- Future work will explore the use of UTMD in patients undergoing catheter-based Y90 radioembolization for unresectable HCC.

Microbubble Cavitation for Improving HCC Radioembolization



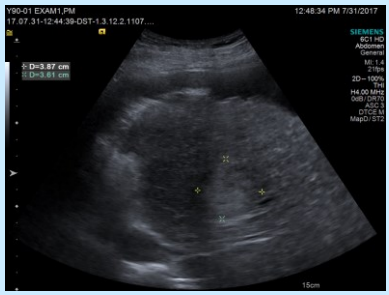
HCV Female Patient Scheduled for Y90 Radioembolization of 3cm HCC In Right Lobe

Microbubble Cavitation for Improving HCC Radioembolization



HCV Female Patient Treated with Y90 Radioembolization of 3cm HCC In Right Lobe

Microbubble Cavitation for Improving HCC Radioembolization



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Microbubble Cavitation for Improving HCC Radioembolization

HCV Female Patient Treated with Y90 Radioembolization of 3cm HCC In Right Lobe

Microbubble applications in radiotherapy

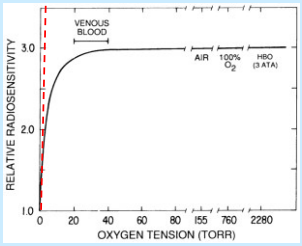
- Microbubble cavitation for sensitizing HCC tumors to radiotherapy
- Microbubble-assisted oxygen delivery for overcoming tumor hypoxia

Background: Tumor Hypoxia

- Angiogenesis in tumors falls short of cellular oxygen demand, resulting in a chronically hypoxic microenvironment.
- Studies have shown this deficit results in cellular resistance to irradiation, which limits the efficacy of radiation therapy.
- A relatively small increase in oxygen partial pressure (pO_2) in hypoxic cells can result in significant sensitization to radiation therapy and this can occur almost instantaneously.
- Approaches using systemic delivery of oxygen immediately prior or during radiotherapy have not translated to clinical usage, primarily due to the body's natural tendency to regulate oxygen.
- Current research is now focused on localized deliver of oxygen for improving radio-sensitivity.

Ultrasound sensitive oxygen-filled microbubbles may be a local, noninvasive, and effective method for overcoming hypoxia-associated radio-resistance

Tumor Hypoxia - Oxygen Delivery

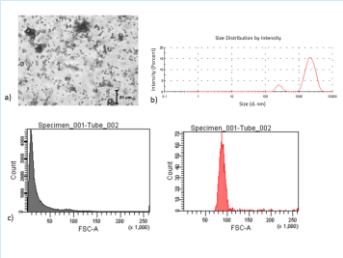


Relationship between relative radiosensitivity and oxygen partial pressure, demonstrating that a relatively small increase in anaerobic tumors can substantially increase sensitivity. Reprinted from [Rockwell 1989].

SE61 Microbubble Fabrication

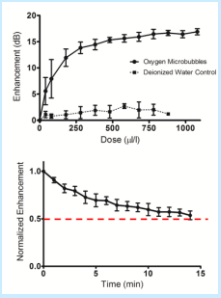
- Span 60 and water soluble Vitamin E (Tocopheryl – α*Polyethylene glycol succinate) are autoclaved before being dispersed in solution.
- The mixture is then purged with octoflouropropane before and during sonication, resulting in microbubble foam.
- The foam is then separated from unincorporated surfactant and repeatedly washed and selected for appropriate size using floatation methods.
- Afterward fabrication these agents can be freeze dried using glucose as a lyoprotectant and their cores re-inflated by venting with the desired gas (Oxygen, Nitrogen, etc).

$SE61_{O2}$ Physical Properties

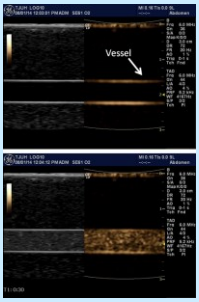


- Average diameter of 3.1 ± 0.1 µm
- Polydispersity index of 0.89 ± 0.18
- Particle counting by flow cytometry showed approximately $6.5 \pm 0.8 \times 10^7$ microbubbles / ml

$SE61_{O2}$ Acoustic Properties



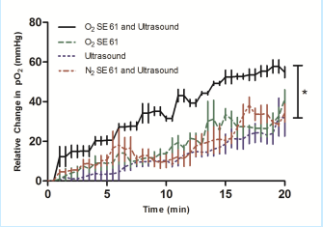
5 MHz Single Element Transducer
(0.45 MPa peak negative pressure)



9 MHz Linear Probe in Contrast Mode
(Logiq 9, GE Healthcare)

$SE61_{O2}$ Oxygen Delivery *in vitro*

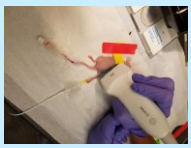
- Oxygen release kinetics were measured using an Oxy Lite 2000 with bare fiber pO_2 probe (Oxford Optronix, Oxford, United Kingdom).
- Two milliliters of reconstituted agent was added to 100 ml of degassed saline.
- Samples were triggered with ultrasound over 20 minutes with readings obtained every 30 seconds.
- All pO_2 values were normalized to baseline levels and expressed as change in mmHg.



*Eisenbrey et al. Int J Pharm. 2015 478:361-7.

$SE61_{O2}$ Oxygen Delivery *in vivo*

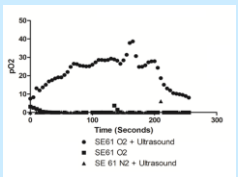
- *In vivo* oxygenation experiments performed in 8 mice with MDA-MB-231 breast tumor xenografts.
- The bare fiber pO_2 probe was introduced into the tumor via a 21G percutaneous catheter.
- Each animal received a 0.1 ml intravenous injection of $SE61_{O2}$ followed by 0.05 ml saline flush during flash-replenishment sequences using an S3000 scanner with a 9L4 probe generating 4 second flash pulses (MI=1.35) with 1 second replenishments (Siemens, Mountain View, CA)
- As controls, release profiles were compared to untriggered $SE61_{O2}$, and triggered $SE61_{N2}$.



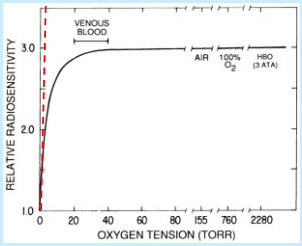
$SE61_{O2}$ Oxygen Delivery *in vivo*

$SE61_{O2}$ Oxygen Delivery *in vivo*

- No changes in pO_2 levels were detected after the injection $SE61_{O2}$ without ultrasound (largest change < 0.5 mmHg) or $SE61_{N2}$ with ultrasound largest change < 3.8 mmHg).
- In tumors treated with $SE61_{O2}$ with ultrasound, pO_2 levels increased in all animals with a peak increase of 22.9 ± 6.4 mmHg.
- Increases in pO_2 levels occurred within 10 seconds and lasted at least 2 minutes in all animals with peak oxygenation achieved 75 ± 28.9 seconds post injection.

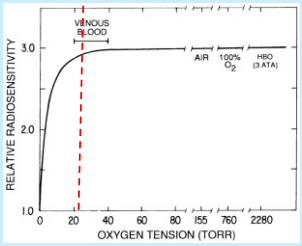


Tumor Hypoxia - Oxygen Delivery



Relationship between relative radiosensitivity and oxygen partial pressure, demonstrating that a relatively small increase in anaerobic tumors can substantially increase sensitivity. Reprinted from [Rockwell 1989].

Tumor Hypoxia - Oxygen Delivery

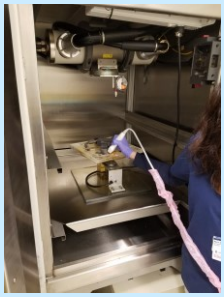


Relationship between relative radiosensitivity and oxygen partial pressure, demonstrating that a relatively small increase in anaerobic tumors can substantially increase sensitivity. Reprinted from [Rockwell 1989].

$SE61_{O2}$ Tumor Radiotherapy Tumor Sensitization

- Radiation therapy experiments underway in 63 mice with MDA-MB-231 breast tumor xenografts.
- Animals receive combinations of $SE61_{O2}$, $SE61_{N2}$, and 75 seconds of ultrasound with or without 5 Gy external beam radiation.
- Tumors growth and survival monitored between groups.

Experimental Design of In Vivo Study			
Group	Radiation Therapy	Microbubble	Ultrasound
1	5 Gy	Oxygen	Yes
2	5 Gy	Oxygen	No
3	0 Gy	Oxygen	Yes
4	5 Gy	Nitrogen	Yes
5	5 Gy	None	Yes



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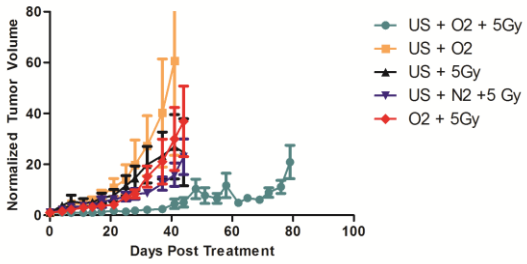

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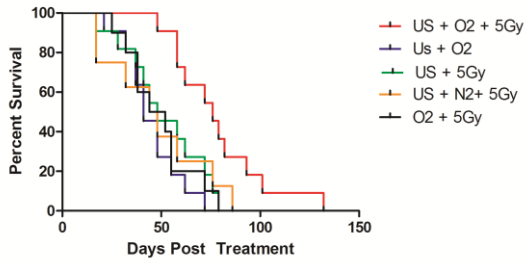

$SE61_{O2}$ Conclusions

- A stable, surfactant shelled, oxygen microbubble has been fabricated.
- Acoustic triggering of the $SE61_{O2}$ bubbles elevates pO_2 levels within 10 seconds and lasted at least 2 minutes with a peak increase of 22.9 mmHg after 75 seconds post injection.
- *In vivo* results indicate ultrasound-triggered oxygen delivery successfully sensitizes breast tumors to radiation therapy, improving both tumor response and survival.

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Thank You!