Advances in molecular biology and cellular biochemistry are providing new opportunities for diagnostic medical imaging to “see” beyond the anatomical manifestations of disease to the earliest biochemical signatures of disease. Molecular imaging generally refers to specific contrast enhancement of biomarkers reflective of underlying pathological processes. This concept is analogous to microscopic detection of specific epitopes with immunohistochemistry techniques translated into a complex and hostile in vivo environment and detected with noninvasive medical imaging systems. Although molecular imaging was once the province of nuclear medicine, today numerous, highly active research programs are found for all clinically relevant noninvasive imaging modalities. Moreover, site-directed molecular imaging systems hybridized with therapeutic agents provide local drug delivery, which can be confirmed and quantified noninvasively at the target of interest. We have developed a novel multi-modal site-targeted contrast agent for sensitive and specific imaging of molecular epitopes and local therapy. This “platform” approach comprises a nanoparticle that is applicable to ultrasound, nuclear, CT, and magnetic resonance. Homing ligands linked to the surface of the nanoparticle bind to cell surface biomarkers to provide high signal amplification. This novel platform has been used to detect angiogenesis, fibrin, tissue factor and collagen III and to locally deliver therapeutic agents through a unique “contact-facilitated” mechanism. Molecular imaging, in conjunction with rational targeted therapies, presents an opportunity with many challenges. Development programs are underway to translate these budding technologies into clinical practice that could likely alter many current clinical paradigms.

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AUGMENTED AND SELECTIVE DELIVERY OF LIQUID PERFLUOROCARBON NANOPARTICLES TO MELANOMA CELLS WITH NONCAVITATIONAL ULTRASOUND

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Our laboratory previously has demonstrated the ability of liquid perfluorocarbon (PFC) nanoparticles to deliver therapeutic agents to cells selectively by binding to specific cellular epitopes, and confirmed the ability to simultaneously image these targeted nanoparticles with ultrasound. In this study, we sought to enhance the delivery of targeted PFC nanoparticles to cells expressing the integrin \( \alpha_v\beta_3 \) (which is involved in angiogenesis in plaque growth and restenosis) using clinical levels of ultrasound energy. This approach would establish the feasibility of using targeted liquid PFC nanoparticles for ultrasonically enhanced noncavitational drug delivery. Nanoparticles complexed with ligands targeted to \( \alpha_v\beta_3 \) were incubated with cells (C32 melanoma) that expressed \( \alpha_v\beta_3 \) in culture. Control nanoparticles were produced that carried no ligand targeted to \( \alpha_v\beta_3 \). A custom specimen holder permitted simultaneous microscopic (Nikon Diaphot 300) visualization of cell interactions during exposure to calibrated levels of ultrasound energy (MI:1.9; exposure time: 5 min; 2-3 MHz phased array transducer: Acuson 3 Va2). PFC content measured by gas chromatography was used as a tracer to confirm delivery of particles to cells.

After nanoparticle binding to cells and application of ultrasound, a >2-fold increase in PFC content of the cells was observed (4.79 ± 0.66 vs. 2.10 ± 0.20 micrograms, with and without ultrasound respectively, \( p<0.005 \)). Control (nonbinding) nanoparticles, ultrasound exposure also increased PFC deposition, but the overall level was substantially less. Videodensitometric data show that nanoparticles were not destroyed by ultrasound exposure. Moreover, the alignment of nanoparticles relative to the incident acoustic field demonstrate conclusively that acoustic radiation forces (primary and secondary) influence the nanoparticles and implicate these forces as participates in the enhanced delivery.

Accordingly, our study shows that enhancement of cellular interaction with targeted nanoparticles is feasible by noncavitational mechanisms. Ultrasonically-enhanced delivery of tracers or drugs to a wide variety of pathologic tissues may be useful for augmenting drug delivery after targeting, while limiting untoward effects on other tissues.
OPTIMIZATION OF SITE-TARGETED
PERFLUOROCARBON NANOPARTICLE CONTRAST IN
WHOLE BLOOD FOR MOLECULAR IMAGING
APPLICATIONS

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The ability to specifically enhance molecular markers of pathology with ultra-
sonic has been previously demonstrated by our group employing a nanoparti-
cle contrast agent. One of the advantages of this agent is its relative non-
echogenicity in the blood pool that allows increased contrast-to-noise between
the blood pool and the bound, site-targeted agent. We sought to define the
contrast agent concentration and acoustic parameters necessary to detect con-
trast enhancement in the blood so that molecular contrast enhancement could
be defined. This study addresses two potential mechanisms that have been pro-
posed for backscatter from the nanoparticle contrast agent in the blood pool
– concentration-related scattering and phase conversion from liquid to gas.
The nanoparticles were produced by methods currently standard in our laboratory using perfluoroctyl bromide (PFOB: b.p. 142°C) as
the major component. Particle size was measured at 200±30 nm. Attenuation
coefficient and backscatter of the agent were measured in whole porcine blood
(hct 40%) and porcine plasma maintained at 37°C. Specimens were insonified
using a broadband, single element transducer (5 MHz, 2.54 cm diameter, 5.08
cm focal length). Acoustic pulses with usable bandwidth of 1.5 to 10 MHz, a
repetition rate of 1kHz, and peak negative pressure of 3.9, 2.7, 1.5, and 0.8MPa
(equivalent to M.I. of: 1.7, 1.2, 0.67, 0.36) were used to measure of attenuation
coefficient and backscatter of nanoparticles at concentrations of 0.26, 0.51, 1.02,
2.04, 4.08 x1014 particles/mL while suspended in either whole porcine blood or
porcine plasma. The attenuation coefficient was linear at all concentrations and
power levels and shows no evidence of a resonant peak characteristic of liquid-
to-gas phase conversion. The backscatter coefficient in plasma increased with
concentration. However, in blood, backscatter was only significantly different
from baseline at 2.04x1014 particles/mL and above (8x the maximum antici-
pated dose). These data indicate that phase conversion of PFOB to gas is not
the source of the contrast in molecular imaging with site targeted nanoparticles.
ULTRASONIC ENHANCEMENT OF $\alpha_v\beta_3$-EXPRESSING CELLS WITH TARGETED CONTRAST AGENTS

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The integrin $\alpha_v\beta_3$ is an adhesion ligand that has been shown to be highly expressed on metastatic tumors and endothelial cells during neovascularization and has been shown to correlate with tumor grade. $\alpha_v\beta_3$ is therefore recognized as a potential binding site for targeted imaging of angiogenesis (the development of new blood vessels required for tumor growth). Flow cytometry is first used to demonstrate expression of $\alpha_v\beta_3$ on A375m melanoma and human umbilical vein endothelial cells (HUVEC). Acoustic backscatter measurements and optical estimates of bubbles/cell are evaluated for these cell layers exposed to three types of targeted contrast agents and two control agents that do not have an attached targeting ligand. Two RGD peptide and one antibody-targeted agent are evaluated, and blocking with free antibody and RGD peptide is used to demonstrate specificity of the agents. Acoustic studies illustrate a backscatter intensity increase from $\alpha_v\beta_3$ expressing cells exposed to the targeted contrast agent from 3 to 20 fold, as compared to controls, depending on cell type, stimulation, and targeting ligand. All three agents successfully and specifically target $\alpha_v\beta_3$. The acoustic signal correlates with the optically determined number of bubbles bound per cell for concentrations less than 1 bubble/cell. Frequency domain analysis demonstrates that adherent targeted bubbles exhibit a significantly more narrowband frequency response than free agents. The mean echo frequency as measured from adherent agents was approximately 2 MHz lower than those from free agents (as determined within the -20 dB bandwidth of the detection transducer). The results suggest that targeted contrast agents may provide additional sensitivity in the detection of tumors, and that adherent contrast agents may be differentiable from free-floating microbubbles.

This research was supported by NIH CA76062, ImaRx Therapeutics, Inc., and an Institutional Research Grant from the UC Davis Cancer Center.

HIGH FREQUENCY NONLINEAR B-SCAN AND COLOR FLOW IMAGING OF MICROBUBBLE CONTRAST AGENTS

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Ultrasound color flow imaging systems operating in the 20-50 MHz range have been developed recently to provide high resolution images of the microvasculature. As with conventional frequency ultrasound, sensitivity to slow flow in small vessels can be limited by issues of signal to noise ratio and tissue motion. We previously demonstrated that substantial amounts of nonlinear scattering from microbubble contrast agents can be stimulated using transmit frequencies in the 14-32 MHz range, which suggested the potential of implementing nonlinear contrast techniques at high frequencies. In this study, we describe the development and evaluation of novel nonlinear microbubble B-scan and flow imaging systems for transmit frequencies above 15 MHz. We first present the results of agent characterization experiments for DefinityTM which confirm nonlinear scattering for the bandwidths and pressure levels employed in this study. Validation experiments using wall-less vessel phantoms show nonlinear B-scan imaging can be achieved using energy in the subharmonic, ultraharmonic, and second harmonic frequency regions for transmit frequencies of 20 and 30 MHz. Both subharmonic and ultraharmonic imaging modes achieved suppression of the tissue signal to below the noise floor, though the SNR in subharmonic mode was 5-10 dB higher than that for ultraharmonic mode. Second harmonic imaging did not result in notable improvements in tissue suppression, due to the substantial amounts of nonlinear propagation present under the conditions that were employed. These effects were explored over a range of transmit bandwidths and pressures. In vivo B-scan experiments using the subharmonic of a 20 MHz transmit show the successful detection of microbubbles in microvessels of the rabbit ear and in the left ventricle of mouse heart, while tissue signals were suppressed to below the noise floor. For color flow imaging, validation experiments using flow phantoms confirm the use of subharmonic velocity and power Doppler. In vivo results in rabbit ear microvessels are shown using the subharmonic of a 20 MHz transmit frequency. The results of this study have demonstrated the feasibility of nonlinear bubble imaging at high frequencies, which may have implications for emerging applications in ophthalmology, dermatology and small animal imaging. This work was supported by the Canadian Institutes of Health Research and the National Cancer Institute of Canada. We thank Bristol-Myers Squibb for providing the Definity.

Session: 2F  
DYNAMIC ELASTICITY IMAGING  
Chair: M. Insana  
University of California-Davis  

2F-1 1:30 p.m.  
A NUMERICAL SIMULATION STUDY OF THREE LINEAR ARRAYS ARRANGEMENTS FOR VIBRO-ACOUSTOGRAPHY  
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