ULTRASONIC TECHNIQUES FOR MOLECULAR IMAGING

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ABSTRACT

Molecular imaging employs the use of site-targeted contrast agents that, once bound, indicate the expression of a molecular signal uniquely associated with a specific disease state. Ultrasonic contrast agents including microbubbles, nanoparticles, and liposomes have been successfully targeted towards angiogenesis associated with tumor growth; inflammation associated with ischemic events; formation of thrombi associated with heart attack and stroke; atherosclerosis; and many other pathologies. Ultrasound has the unique property of being able to interact with tissue while simultaneously visualizing anatomical features and function. This ability makes ultrasound a unique tool for both visualization of pathology and for the potential delivery of drugs to specific locations within the body.

1. INTRODUCTION

1.1 Ultrasonic Molecular Imaging

Molecular imaging refers to a broad class of applications extending imaging modalities from their traditional roles for morphological and functional imaging to the detection and spatial location of biomarkers that are below the physical resolution of each imaging system. These biomarkers are often the first signs of an impending disease state and if detected early may allow for therapeutic intervention before serious symptoms appear. Ultrasonic molecular imaging is a relatively recent addition to the field of molecular imaging with specific applications to the detection of thrombus, the measurement of neo-vasculature around a tumor, and the determination of regions of inflammation. Ultrasound has several advantages over other imaging modalities in its ubiquity, cost-effectiveness, portability, and real-time nature. The ability to use ultrasound to deposit focused energy to remote tissue within the body makes possible the area of "theranostics" or the potential of simultaneous diagnostic testing and therapy.

1.2 Ultrasonic Contrast Agents

Ultrasound molecular imaging relies upon the use of contrast agents to enhance regions of pathology. The traditional ultrasonic contrast agents for blood pool applications are microbubbles. These are gaseous particles typically on the order of 2 to 4 microns in diameter. A shell surrounds the microbubbles in order to stabilize them within the blood pool, to prevent or reduce unwanted interaction with the immune system, and to alter the acoustic properties of the contrast agent. Currently in commercial use, there exist several formulations of contrast agents each with a unique gas and shell material formulation. A second type of contrast agent is currently being examined that uses perflurocarbon liquid nanoparticles. These particles are approximately 250 nm in size. One of the specific characteristics of both approaches for ultrasonic contrast agents is the relatively large size in comparison to the contrast agents in other imaging modalities (MRI – Gd particles, PET/SPECT – radioactive tracers). This larger size limits the ultrasonic contrast agents to the blood pool. Therefore, any detection of contrast agent by ultrasound can reliably be classified as a targeted pathology in contact with the blood pool.

1.3 Detection Challenges

Ultrasonic molecular imaging has many challenges to the detection of pathology. Some of these challenges are shared with other imaging modalities such as the availability of receptors on the targeted pathology, biological variation, and the specificity of the biomarker. Ultrasonic imaging also has its own unique challenges. For microbubbles, these interesting research problems include the determination of site-targeted contrast agent in circulation from that bound to a specifically targeted site, the differentiation of contrast agent from tissue, and the limited lifetime of the contrast agent in circulation. For nanoparticles, a different set of challenges exist which include the detection of low amplitude scattering particles in the midst of high amplitude scattering from tissue.

Although the field of ultrasonic molecular imaging is relatively new, there are too many studies to summarize within this paper. In order to illustrate some of the problems and novel solutions in this area, we will focus on two specific approaches in the next section. These examples serve to show the work from two

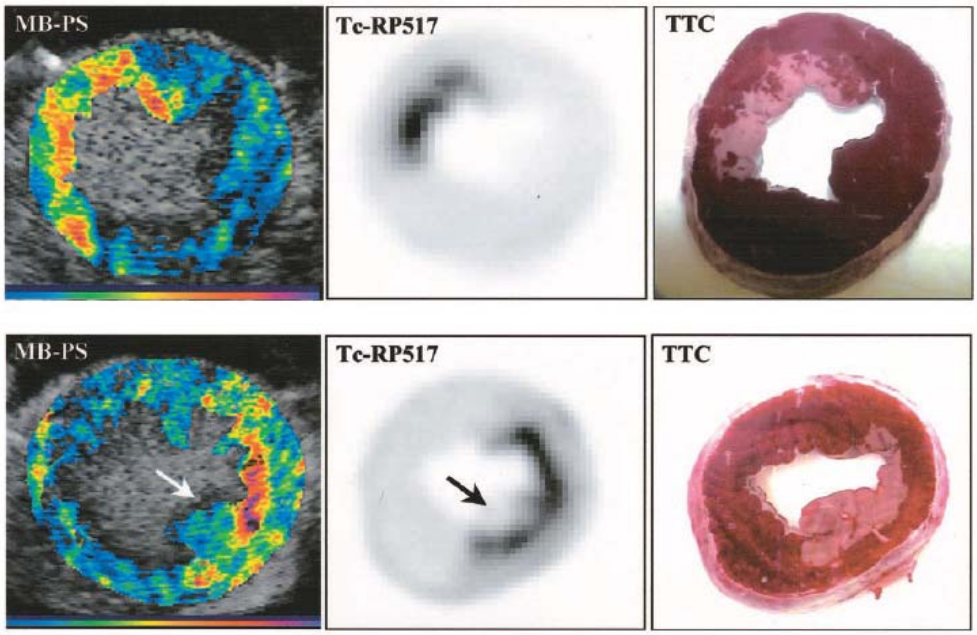

Figure 1: The left panel shows microbubbles targeted to inflammation in canine myocardium during ischemic injury. The center panel illustrates the same heart with a nuclear perfusion study. The right panel shows the excised tissue stained for infarcted tissue. (reprinted with authors' permission from ***Circulation*** **2002;105:1764-1767**)

laboratories using both the approach of nanoparticle and microbubble targeting.

2. METHODS

2.1 Perfusion Detection Methods

It may be helpful to the reader to provide a very brief summary of the detection methods used for ultrasonic blood pool contrast agents – specifically microbubbles. Microbubbles as have been described above are stabilized gas pockets. The gas bubble is of a specific size that possesses a resonance frequency in the center of typical ultrasonic diagnostic frequency range. This resonance behavior was the initial reason for the choice of microbubbles as contrast agents and resulted in a quite large or bright signal on an ultrasonic image. However, the utility of this approach was limited as it was difficult to separate the signal from contrast agent and that returned by interaction of the insonifying beam with tissue. Several approaches were then fashioned to overcome this limitation. A good summary of these techniques can be found in Averkiou et al. [1] In brief, the bubbles behave in a non-linear manner when interacting with the ultrasonic wave. This non-linear behavior was exploited in a variety of ways to differentiate contrast agent from tissue. One approach is called "harmonic" imaging and is the result of frequency domain filtering to separate the fundamental frequency from the first harmonic. Variations of this approach have been used to separate the fundamental frequency from the 2nd and higher harmonics, and also the fundamental from the sub-harmonic frequencies. A second approach is to utilize the asymmetric interaction of ultrasound with a bubble during the compression and rarefaction phases. By sending two pulses, each an exact inversion of the other, the scattered echoes for tissue will be exact inversions of one another while the echoes from bubbles will have differences depending on the order of compression / rarefaction. By combining the echoes from the two pulses, the tissue signal should cancel leaving only the even harmonic terms. A third approach uses a technique known as "power modulation" which utilizes the fact that bubbles respond with a different degree of non-linearly depending on the driving amplitude. By sending an initial signal at half the amplitude of a second signal and then subtracting twice the first echo signal from the second echo signal, it is possible to differentiate contrast signal from tissue. Ideally, the contrast signal should not scale in proportional to the insonifying field and therefore the subtraction yields a remaining portion that is characteristic of the non-linearity of the bubble. A fourth methodology is common for microbubble imaging which uses the fact that the contrast agent is sensitive to high-pressure acoustic waves. With this insonification scheme, it is possible to destroy all contrast agent in the field of view. This allows an effective "reset" switch that zeroes the presence of contrast agent in the image and allows for imaging of re-perfusion of the organ. These methods and variations on these approaches are the primary detection methods for microbubble agents and may be applied and extended to the realm of ultrasonic molecular imaging.

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2.2 Microbubbles

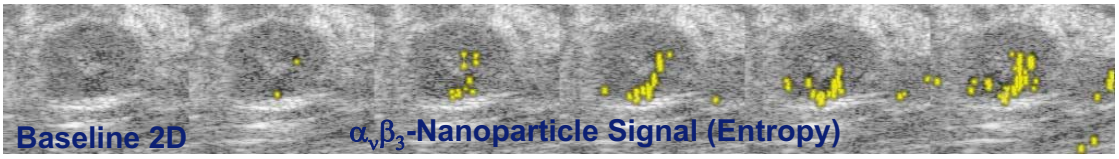

Figure 2: Clinical images from an in vivo rabbit model with an implanted cancer. The yellow represents signal entropy exceeding a constant threshold for all images. The images from left to right represent baseline, 0, 15, 30, 60, and 120 minutes post-injection. (printed with authors' permission)

Lindner et al. have used microbubbles that have been specially formulated to target specific pathologies. In particular, they have targeted inflammation through passive means by using the immune interaction between charged bubbles and the regions of inflammation (Figure 1).[2] They have also looked at active targeting of inflammation by attaching proteins that interact with P-selectin, a protein expressed in the inflammation response. In a separate study, the group has used microbubbles that are modified to specifically target an integrin expressed in regions of neo-vasculature ($\alpha_v\beta_3$).[3]

The technique that Lindner's group has developed for measuring targeted microbubbles makes use of the physiology of the animal, the sensitivity of contrast agent to high pressure fields and the knowledge that the ultrasonic contrast agent remains in the blood pool. A summary of the technique follows: The contrast agent is injected into the animal and allowed to circulate. After a pre-determined time period, the contrast agent has bound to the targeted receptors, but there is also contrast agent freely circulating in the blood pool. To differentiate these two states of contrast agent, a low MI (mechanical index – a measure of the peak negative pressure divided by the square root of the frequency) interrogating pulse is sent into the tissue. The reconstructed image contains contrast agent that is both bound to the pathology and that remaining in circulation. A second high MI pulse is sent into the tissue to destroy all contrast agent within the field of view. A specific short time period elapses during which contrast agent washes back into the tissue but does not yet have time to bind in sufficient quantity. A third low MI pulse is then used to insonify the tissue that contains ideally only freely circulating contrast agent. By subtracting the image made from the first pulse from that in the third pulse, the difference image represents the areas with bound bubbles.

From the same laboratory, Klibanov et al. have shown that the binding of microbubble agents can be enhanced by exposure to acoustic radiation force. The force exerted on the bubbles moves the particles into proximity with the sides of the vessel wall. This proximity increases the chance of microbubbles binding and can thereby enhance the overall signal. [4]

Several challenges remain for this approach. One obvious problem occurs from the use of an image subtraction approach that is the registration of the three imaging planes. This requires that the ultrasonic probe remain fixed in location with respect to the imaging plane on the patient. This can be difficult in practice although not insurmountable. Another problem occurs if there is imperfect separation of contrast and tissue signals, especially in the case of small-detected contrast signals. There are other experimental difficulties that include the effect of ultrasonic attenuation due to overlying contrast-perfused tissue. Despite these challenges, the approaches taken by this laboratory show very promising results.

2.3 Nanoparticles

Wickline et al. have demonstrated the utility of using nanoparticles for enhancing several pathologies. In particular, the group has shown the enhancement of plasma clot in vitro and in vivo, tissue factor in vivo, and recently shown the potential of enhancement of neo-vasculature associated with cancer (Figure 2).[5] The nanoparticle contrast agent can be useful for targeting many pathologies accessible through the blood pool. Although this contrast agent has many advantages such as a low signal in the blood pool (which limits the background signal), it also poses some unique challenges for detection. The method of interaction of these particles with ultrasound is posited to be a simple transmission line acoustic reflection model. This explanation means that the signal received from the nanoparticles is the exact same mechanism of sound interacting with other acoustic impedance mismatches in the body. As a result, it can be very difficult to determine which portion of the signal is from tissue and which is from the targeted nanoparticles.

Wickline et al. have used several approaches to differentiate targeted tissue from normal tissue. The simplest approach has been to characterize the time course change in the acoustic signal energy returned from a surface. Such an approach relies on the ability to examine a specific volume of tissue over the course of injection and the binding phase (a process that can take upwards of

> 2 hours). Because of the lengthy time scale, and the unlikely ability to return to the exact tissue volume at a later time period, this approach is limited in its application. A second approach applied by Hughes et al. has the goal of increasing the contrast signal to background signal. This approach uses the reduction of the ultrasonic signal to its information content. Hughes et al. applies information theoretic parameters through the use of various discrete and continuous entropy metrics.[6] This approach attempts to improve delineation of contrast agent and tissue.

Although many of the approaches employed for the detection of nanoparticles show promise in specific limited pre-clinical settings, there still remain several challenges. The primary challenge is to determine if there is a unique acoustic signature from the presence of nanoparticles bound to the surface of pathology that allows for delineation of the pathology in a "one-shot" ultrasound approach. In other words, an approach is desired that allows the clinician to image the patient after injection of contrast agent and to arrive at an unambiguous decision of the presence of signal from a targeted region. Many of the same challenges that apply to the microbubble approaches also apply in the current setting, including the need for good spatial registration of images during the binding.

3. DISCUSSION

This paper summarizes the state of the art in molecular imaging with ultrasound through the inclusion of two examples of laboratories with successful studies. In both cases, the technology is currently at the pre-clinical stage and needs to solve several major challenges to demonstrate robustness necessary for clinical utility. The investigators employ a clever combination of variations in the transmitted signal and received signal processing to attempt to separate the unique signature of specifically targeted particles.

There are many other laboratories engaged in the pursuit of ultrasonic molecular imaging. Unger et al. has shown successful binding of microbubble contrast agent to fibrin in an atrial clot model.[7] Dayton et al. have shown binding of contrast agent to $\alpha_v\beta_3$ integrin.[8] Villanueva showed binding of anti-CAM-1 labeled microbubble contrast agents to activated endothelial cells.[9] These and other ongoing studies are discovering new methods and applications for ultrasonic molecular imaging.

There remain several important signal-processing challenges to solve to increase the utility of ultrasonic molecular imaging. These challenges include the detection of potentially low amplitude contrast-derived signals with respect to tissue signal as in the case of nanoparticle agents. An increase in the sensitivity of detection for bound microbubble contrast agent to the large signal from freely circulating contrast agent is a necessary step for many applications.

4. REFERENCES

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