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Future of molecular imaging and role of nanotechnology and chemistry in ultrasound method for disease treatment

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ABSTRACT

Resembling most other techniques for medical imaging, ultrasound (US) changed radically that have ameliorate its spatial and temporal resolution, provided three-dimensional data, and permitted evaluation of dynamic physiologic processes similar to blood flow or tissue motion. The possibility of targeting US conflict agents to disease-related markers and for non-invasively evaluating the molecular profile of disease in animal models was strongly established. The technique is now being applied as a high-throughput research tool that can define pathophysiological processes in both the spatial and temporal domains. Early steps are presently under achievement in the direction of the advance of targeted agents appropriate for clinical use of this useful method.

Keywords: molecular imaging, nanotechnology, ultrasound, medical imaging, MRI

1. INTRODUCTION

The past decades have seen momentous developments in noninvasive medical imaging. Similar to most of systems for medical imaging, ultrasound (US) has undergone radical changes that have enhanced its spatial and temporal resolution, provided three-dimensional data, and permitted evaluation of dynamic physiologic processes for example: blood flow or tissue motion. This method is extensively used cross-sectional imaging modality worldwide. Important recent developments include MB contrast agents, quantitative approaches and new signal processing and display methods. Ultrasound is the sound at a frequency higher than 20 kHz, and may be crashed by the frequencies used. Clinical diagnostic ultrasound scanners use frequencies from 1 MHz to 20 MHz. One current advance has been the capacity to use molecular imaging (MI) to measure tissue phenotype, enzymatic movement or gene expression. Even though methods for MI have been advanced for in effect every arrangement of medical imaging, there are substantial differences that inspiration the optimal of imaging method in both the research and clinical settings. E.g., alterations in the recognition technique (e.g. US, gamma radioactivity indicator, magnetic resonance, visual light, etc.) Inspiration spatial determination, sensitivity and practicality for use. Alternatively, changes in the imaging probe effect virtual toxicity, biodistribution, sensitivity, specificity, and temporal resolution. High sensitivity, availability, rapid execution of imaging protocols, and the comparatively low rate are features of targeted MI with US that make this technique attractive particularly for screening large patient groups for a potential disease. US contrast agents have been in clinical use for years for applications. For example blood pool improvement, characterization of liver scratches or perfusion imaging. These contrast agents are usually in the form of small acoustically active particles reaching from several hundred nanometers to a few micrometers in width. For clinical imaging, all contrast agents in current use are top secret as microbubbles (MBs) having a mean diameter of >1 mm. The principle behind using MBs such as US contrast agents is created on their compressibility. Gas-containing MBs are numerous tips of magnitude more compressible than water or tissue, and are smaller than the wavelength of the applied US field in the diagnostic frequency range. Currently, US imaging generally plays a minor role in MI. Most of the clinical actions are attentive on PET and SPECT, and most of preclinical research is extended to the areas of optical imaging and MRI. However, with proper contrast agents designed for MI, US can play an essential role both in research and clinical applications. Historically, US contrast imaging was recommended for visualization of blood flow patterns.

Basic mechanism of contrast agent in US: Early-generation US contrast agents included air bubbles equipped by fast mixing air with aqueous saline, albumin or X-ray difference media instantaneously (seconds) before intravascular administration. Air bubbles were echogenic but would rapidly dissolve in the blood; bubbles would not pass through the pulmonary circulation, hence monitoring blood flow subsequent intravenous management had not been feasible with those contrast particles. Other sometimes exotic, MB preparation approaches were investigated, such as intravenous administration of H2O2 to generate oxygen bubbles in vivo, but they did not gain widespread use. Next, so-called first-generation contrast supplies were much more steady and circulated longer than uncoated air bubbles because they were stabilized with a surfactant or a protein shell. They were able to trans pulmonary passage, so blood flow characterization after intravenous administration became possible, though circulation time of air-filled bubbles was generally quite short and these contrast agents did not gain much recognition in clinical use. Important clinical use of MBs for blood pool improvement became likely less than a decade ago, with the agreement

of second-generation contrast agents, which contained water- unsolvable fluorinated gases. These resources stayed in the bloodstream for minutes, not seconds, so, viable in vivo imaging studies have been performed. Particle size constraints apply to the design and applicability of MI with US contrast agents to attain successful imaging. In most cases, these resources are gas-filled MBs with the size of numerous microns. These subdivisions, with a picogram mass, are noticed by US very well, to the level of imaging separate particles in the adjourned public and even after binding to the target. Submicron gas bubbles keep much worse strength and circulation time; they are not as echogenic as larger bubbles. Therefore, favored contrast particle size is 2-4 um (larger MBs may lodge in the capillaries, e.g., in the lungs). It means that successful MI with present generation of MB contrast agents is thinkable only for intravascular markers, and US contrast detection of a precise cellular marker is not possible if that marker is not visible to the bloodstream and is located at the interstitial space or inside the target cell.

Role of MB in MI: 15 years ago, dozens of papers describing MI with US contrast have appeared, presenting targeting to a variety of the disease marker molecules. General design of the particle has not changed suggestively since its first presentation. Targeting ligand is involved to the MB shell-forming substantial, then MBs are produced from inexplicable gas during high-shear mixing, e.g., with a probe-type sonicator. During the preparation procedure, MBs are instantaneously roofed with the shell. After removal of excess ligand by centrifugal flotation, MB targeting is either tested in vitro or injected intravenously in the experimental animals with the appropriate disease models. Most successful and popular targeted MB shell design is based on a lipid monolayer shell decorated with a grafted brush of the water-soluble polyethylene glycol. The depth of the lipid monolayer is ~2 nm. This tinny shell does not delay significantly with MB increase and reduction in the US field, providing good contrast echogenicity without the need to destroy the particles by blowing up the shell with great-power US. This shell does not attend as a significant barrier for gas diffusion in and out of the bubble. PEG chains extend from the MB surface into the surrounding aqueous environment by ~10-30 nm, which is not very important when compared with a 2-3 um particle diameter. Targeting ligand is usually attached to the exterior of the PEG brush in the same extended "fishing line" manner as it was earlier proposed for liposomes. This ligand coupling technique enables ligand-receptor interface and improves microparticle targeting in a flow-through scenario.

Peptides/mimetics, carbohydrates, glycosulfopeptides, small and large proteins (such as antibodies) were successfully tested as ligands for MI using US. Most of tests like this, required biotinylated ligand which would assign to the bubble via a streptavidin spacer; many biotinylated ligands are available commercially or can be equipped easily. The advantage of biotin-streptavidin ligand linking method is in its simplicity; bubbles can be easily centrifuged to remove excess of free ligand. This approach is particularly useful if the ligand is a large protein that could become denatured and inactivated during MB preparation by sonication as labelled in the previous paragraph. However, there is a very important disadvantage. Streptavidin, as an uncommon protein, will not be agreed for clinical application in human setting. Therefore, chemical coupling amongst ligand and shell has to take place, either before (as described above) or after bubble preparation. The choice of ligands for MB targeting may be determined not just by the specificity and affinity of ligand-receptor interaction for the respective molecular markers (thermodynamics), but also by how fast the ligand-receptor interaction takes place (kinetics). Antibodies are usually selected via ELISA, by the value of equilibrium affinity, often described as equilibrium dissociation constant; however, the act of interaction of ligand-MB with the receptor on the vessel wall is a rather time constrained event, and if the kinetics of friendship between the ligand and receptor is slow, the MB would be transported away from the target area and would not assign to the intended target. However, successful particle targeting is possible via a mixture of speedy association between the ligand and receptor, and cooperative multipoint interaction between great number of ligand and receptor molecules. Placing over a million small ligand molecules per single MB is feasible. Only ~105 (or less) antibody molecules committed per MBs is typically reported. Using full IgG molecule for immobilization on the MB surface may not be the best strategy as compared with the use of smaller ligands: larger molecules carry much more than just the target binding sequence.

Fc fragment of the antibody may be quite immunogenic, so the use of humanized (or fully human) antibodies is required. Furthermore, Fc receptors on phagocytic cells (e.g., in the liver) may alter mb biodistribution in an undesirable way. Overall, the use of smaller molecules as ligands for MI using US should be preferred. There are numerous useful intravascular molecular targets that mark disease tissue location; most of them are affiliated, directly or indirectly, with endothelial cells, and constitute markers of inflammation or angiogenesis. Imaging procedure is pretty straightforward, but somewhat different when compared with other MI modalities. Targeted MB construct aqueous dispersion (or control non-targeted preparation) is injected intravenously as a bolus, and low-power contrast US imaging of the region of interest is performed immediately. Contrast inflow imaging provides real-time information of the blood flow patterns in the tissue of interest, and may be useful for immediate understanding of the target tissue physiology. Numerous minutes later, imaging of MBs that have knotted in the tissue can be performed. By that time, repeated circulation of the injected MBs through the body vasculature results in the selective binding of a fraction of the injected bubbles to the target, e.g., disease tissue. The majority of contrast agent is not likely to

adhere to the molecular marker of disease and is recirculating with the blood flow. MB shell is too thin to be a respectable barrier for air diffusion into the bubble and fluorocarbon diffusion out of the bubble. As MB contrast passes through the lungs, gas exchange with the surrounding medium takes place; most of the fluorocarbon gas is swapped out of the bubbles and exhaled within minutes of intravenous management. Residual air in the bubbles does not support good stability in vivo, it diffuses out and shell collapses, making the particle non-echogenic.

Targeted bubbles, adhered e.g., to tumor vasculature, are not uncovered to fast flow convection conditions that support speedy gas exchange during circulation, so we can hypothesize that loss of gas from targeted bubbles would be slower. This method can contribute to the improvement of signalto- noise (e.g., target-to-blood) localization ratio. So at 5-15 minutes after MB administration, more of the circulating bubbles have cleared from the bloodstream, and imaging of adhered bubble can be achieved. Modern contrast modes (such as Contrast Pulse Sequencing or Pulse Inversion Amplitude Modulation) implemented on many US imaging systems allow to suppress the signal from the tissue itself, and observe MB with excellent detection sensitivity in real time at low power without contrast destruction (US Mechanical Index <0.2). After observing the targeted bubble in the particular imaging plane(s), US power can be increased, and adhered bubbles destroyed. Decrease of the acoustic backscatter signal after destructive pulse can serve as an additional proof of MB targeting; within seconds after reduction of Mechanical Index, we can observe residual circulating bubbles still present in the bloodstream.

DISCUSSION

Nearly all of the MI using US studies defined in the literature so far has been performed in rodent animal models. There is an exception—a clinically approved agent that is routinely used for Kupffer cell targeting, Sonazoid (perflubutane MBs). It is approved for targeted US contrast in the liver radiology setting in Japan. It is likely that several hundred thousand patients have received this contrast agent since its approval. Sonazoid increase in normal liver parenchyma is achieved via phosphatidylserine targeting: Sonazoid MB consists of phosphatidylserine shell and perfluorobutane gas. As phosphatidylserine is the natural marker of apoptosis, its presence on the outer surface of a particle or a cell makes that surface an immediate target for phagocytic uptake. In the normal liver tissue but not in the lesion foci, Kupffer cells are present and can capture circulating MBs and allow better disease characterization by "negative" target enhancement, as compared with unenhanced US or dynamic CT. This "late-phase" US imaging is achieved to detect bubbles that have targeted to the normal liver tissue of interest following the clearance of circulating bubbles from the bloodstream, several minutes after bolus intravenous administration. As phosphatidylserine-targeted MBs are taken up by any phagocytic cells, they may be applied not only for liver radiology, but also for imaging of adherent neutrophils in the acute inflammation or ischemia/reperfusion injury scenarios.

Generally, two strategies are generally employed for the targeting of MBs. The first involves modifying the constituents of the MB shell to facilitate their addition to cells encountered in disease areas, such as leucocytes. One example of this tactic is the incorporation of the negatively charged phospholipid phospatidylserine into the shell, which promotes activation and adhesion of complement to the MB shell and thus promotes MB attachment to activated leukocytes. Similarly, MBs with an albumin shell can attach to leukocytes through cell-surface receptors that bind albumin such as integrins. The second general targeting strategy consists of attaching disease-specific ligands, such as monoclonal antibodies, glycoproteins, carbohydrates, peptides or peptidomimetics to the MB shell. The ligands are conjugated either directly to the shell or involved to a polyethyleneglycol spacingmoiety that schemes the ligand away from the shell surface. Both covalent binding with maleimide or biotin– avidin links can be employed to attach the ligands to the MB shell. In general, several thousand binding molecules per square micron shell surface area can be conjugated. For successful targeting of a disease-related molecule, several factors have to be considered. The targeted molecule should be sufficiently particular for the disease course and should not be expressed constitutively in significant amounts.

Attachment of MBs happens in the face of vascular flow, and thus factors like shear rate in the tissue of interest, ligand density on the MBs, bond affinity of the ligand (on- and off-rates), and target molecule density have to be considered. Up currently, the principle of targeting acoustically active particles for imaging disease has been established. A wide multiplicity of contrast agents and probes are currently in routine use in experimental setups. Unlike other MI techniques, particularly radionuclide based methods; MI with US is cheap, widely available, and easy to perform and has a good spatial and temporal resolution. It is a quantitative technique with high sensitivity and good signal-to-noise ratio. Despite of these advantages, further steps have to be made to facilitate its clinical application: optimization of MB adhesion to target structures is needed to further improve signal to noise ration of the technique. More disease specific targets will widen its clinical spectrum of applications. Imaging techniques could be improved by using new detection algorithms, considering the specific changes in acoustic assets of MBs when binding to a target. Although ultrasonic molecular imaging has made important growth over the last decade, this technology still faces several challenges before it can rise to its full diagnostic potential. It is the ideal goal of this technology to determine if a molecular target is present and, if so, to what degree. This involves that the contrast

agents explicitly adhere to their molecular target and bind in quantities great enough to overwhelm the signal contributions from nonspecific retention. Additionally, the ultrasound system should have sufficient sensitivity to detect the targeted agents present at the site of pathology and be able to measure the pathology in its entirety. In this review, we hypothesize that numerous limitations have slowed the development of ultrasonic molecular imaging; however, current advances in contrast agent development, ultrasound technology, and detection strategies demonstrate the probable to substantially improve the capabilities and utility of ultrasonic molecular imaging. We review these challenges and current advances in the following sections.

2. CONCLUSION

Targeted contrast-enhanced US is an emerging imaging. Strategy that syndicates US technology with novel molecularly targeted US. Contrast agents for assessing biological processes at the molecular level. US contrast agents are nano or micro-sized particles that are targeted to particular molecular markers by adding high-affinity binding ligands on to the surface of the particles. MI with US contrast agents represents a rapidly developing area of research. A combination of equipment portability, low cost and excellent contrast detection sensitivity by US imaging, in concert with the ability to detect molecular markers of disease in real time, will very likely lead to the development of MI contrast agents for widespread clinical application for diagnostic imaging and image-guided therapy. In conclusion, the possibility of directing US contrast causes to disease-related markers and for non-invasively evaluating the molecular profile of disease in animal models has been resolutely established. The technique is now being applied as a high-throughput research tool that can define pathophysiological processes in both the spatial and temporal domains. Early steps are presently being made in the direction of the development of embattled agents appropriate for clinical application of this useful method. Following intravenous administration, these targeted US contrast agents accumulate at tissue sites overexpressing specific molecular markers, thereby enhancing the US imaging signal. High spatial and temporal resolution, real time imaging, non-invasiveness, relatively low costs, lack of ionizing irradiation and wide availability of US systems are advantages compared to other MI modalities.

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