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Molecular imaging agents for ultrasound

Aimen Zlitni[1] and Sanjiv S Gambhir[1,2]

Ultrasound (US) imaging is a safe, sensitive and affordable imaging modality with a wide usage in the clinic. US signal can be further enhanced by using echogenic contrast agents (UCAs) which amplify the US signal. Developments in UCAs which are targeted to sites of disease allow the use of US imaging to provide molecular information. Unfortunately, traditional UCAs are too large to leave the vascular space limiting the application of molecular US to intravascular markers. In this mini review, we highlight the most recent reports on the application of molecular US imaging in the clinic and summarize the latest nanoparticle platforms used to develop nUCAs. We believe that the highlighted technologies will have a great impact on the evolution of the US imaging field.

Addresses
[1] Department of Radiology, Molecular Imaging Program at Stanford, Canary Center at Stanford for Cancer Early Detection, Stanford University, Stanford, CA, United States
[2] Department of Bioengineering, Department of Materials Science and Engineering, Stanford University, Stanford, CA, United States

Corresponding author:

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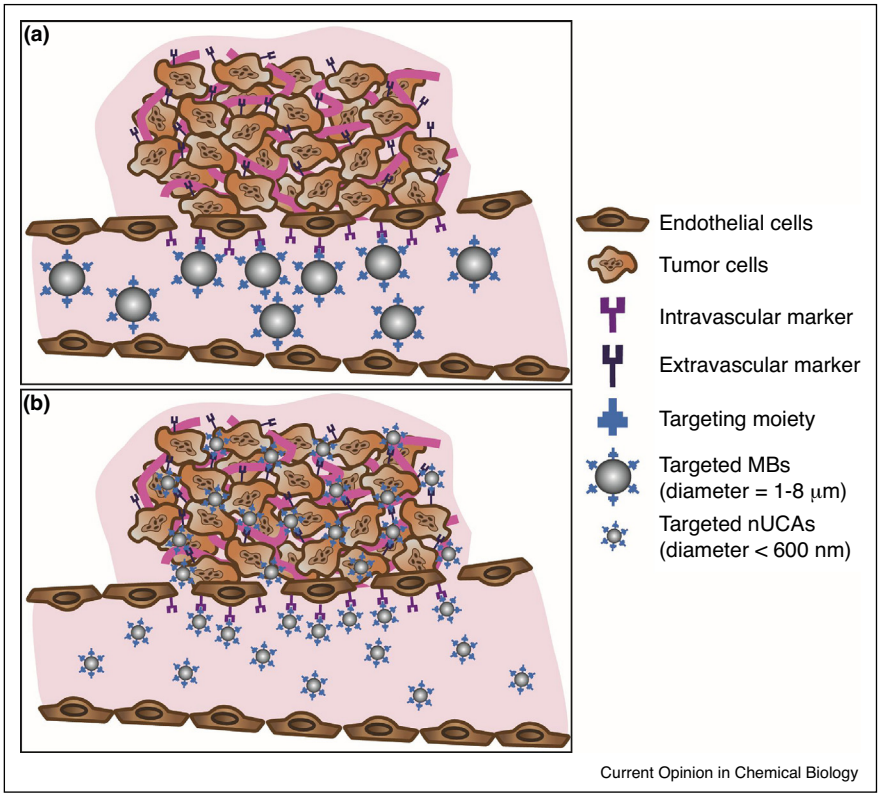
Introduction

Ultrasound imaging (US) is the second most widely used medical imaging modality due to its high sensitivity, portability, relative low cost, and good safety profile (no ionizing radiation) [1]. In US imaging, anatomical images are produced after measuring the propagation of high frequency sound waves when travelling between materials and tissue interfaces of different acoustic properties [1]. Contrast enhanced ultrasound (CEUS) takes advantage of using highly echogenic contrast agents to further amplify the US signal allowing imaging organs with low US contrast (i.e. blood pool) [2]. Ultrasound contrast agents (UCAs) are typically gas-filled micron-sized bubbles (MBs) with a 1–8 μm diameter which oscillate upon interacting with the US wave thus enhancing the reflected US signal. To date, seven MBs have been approved for clinical use and are routinely utilized to improve organ imaging and to better assess blood flow and vascularization [2–4]. In the past two decades, many efforts focused on developing MBs which are targeted to vascular endothelial markers of disease by attaching biomolecules to its surface expanding the utility of CEUS in the molecular imaging field [5•]. Preclinical validation of molecular US imaging of a variety of diseases such as inflammation (inflammatory bowel disease, myocardial ischemia, atherosclerosis and cardiac transplant rejection) and cancer (pancreatic, angiosarcoma, ovarian, prostate, breast, colon, liver, renal, glioma and melanoma) have been reported [5•]. In addition, advances in transducer technology and pulse sequences stimulated the use of CEUS for therapy through ultrasound-mediated bubble destruction. Such innovations are now widely employed to reversibly break the blood–brain barrier, cause cavitation and enhance site specific drug/gene delivery with improved therapeutic outcomes [6]. Unfortunately, current UCAs cannot extravasate beyond the vasculature due to their micron-size and have limited circulation time constraining the advancement of molecular US imaging (Figure 1a). In this review, the first application of targeted MBs for molecular US imaging of disease in the clinic will be summarized and recent efforts in the development of the next generation of nano-sized UCAs (nUCAs) will be highlighted.

Molecular US imaging in the clinic

To date there is only one molecularly targeted UCA that is being evaluated in the clinic. This contrast agent, named BR55 (Bracco, Italy), has a mean diameter of 1.5 μm and is composed of a phospholipid shell and a gas core consisting of perfluorobutane and nitrogen. BR55 is targeted to kinase insert domain receptor (KDR) which is a human analog of vascular endothelial growth factor receptor type 2 (VEGFR2) a known marker overexpressed in many human cancer types [7]. After an extensive preclinical evaluation of BR55 by various groups and in multiple animal models, BR55 is now being evaluated in humans for prostate, ovarian, pancreatic, and breast cancer imaging. Our group published their first in human results showing the efficacy and safety of BR55 in evaluating patients with breast (n = 21) and ovarian (n = 24) cancer lesions [8••]. After BR55 injection and ultrasound imaging for up to 29 min, tissue samples were taken out in order to correlate the KDR-expression observed indirectly via the US image with that observed through direct immunohistochemistry (IHC) staining. The US image signal matched well with KDR-expression on IHC (93% of breast and 85% of ovarian malignant lesions). The strong KDR-targeted US signal was present in 77%

Figure 1



Schematic representation of molecular US imaging using micro-sized and nano-sized UCAs (MBs and nUCAs respectively). **(a)** Molecular US imaging using targeted MBs which are limited to the vascular space and can only target intravascular markers of cancer due to inability to extravasate into tumor microenvironment. **(b)** Molecular US imaging using targeted nUCAs which can actively target intravascular and extravascular markers of cancer as well as passively accumulate in the tumor microenvironment through the enhanced pore and retention effect (EPR).

and 93% of ovarian and breast malignant lesions respectively. Although not designed to measure accuracy, the results were encouraging for further continued testing. Smeenge and co-workers recently conducted a phase 0 study in prostate cancer (PCa) patients to assess the feasibility and safety of BR55 in detecting PCa lesions [9]. Upon improving scanning settings, 68% of PCa lesions detected by histology after prostatectomy were localized through BR55 US imaging ($n = 12$). These pilot results show promise in the utility of US molecular imaging in differentiating between malignant and benign lesions making it an important tool to help reduce unnecessary biopsies or surgeries in the future.

Nano-sized UCAs (nUCAs)

One major limitation in targeted molecular US imaging is the lack of submicron-size UCAs which can still amplify the US contrast as well as extravasate beyond the vasculature to target disease biomarkers (e.g. on tumor cells) directly (Figure 1b). Unfortunately, reducing the size of the MBs not only reduces its echogenicity under clinical ultrasound but also reduces the stability of gas-filled bubbles in solution [10]. This makes it extremely challenging to develop small yet highly echogenic particles resulting in the need to exploit other non-traditional strategies to develop nUCAs. A variety of nUCAs have been developed/discovered which enabled new, paradigm shifting applications of CEUS in diagnosis and therapy (theranostics) [11–14]. Such agents include chemical-based agents (developed from organic and/or inorganic material) as well as genetically engineered protein-shelled particles (such as gas vesicles) [15,16••]. A wide range of echogenic nUCAs with an inorganic shell have been reported and are reviewed elsewhere [12,17••,18•]. The following sections will highlight novel efforts (2015-present) in developing organic nUCAs. While many of these agents are employed for both imaging and therapeutic applications, the focus of this review will be more towards their utility and potential as molecular ultrasound imaging agents.

Organic nUCAs

The advancements in nanomedicine resulted in a rapid development of echogenic and biocompatible organic