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● *Technical Note*

EVALUATION OF THE SPECIFIC ADSORPTION OF BIOTINYLATED MICROBUBBLES USING A QUARTZ CRYSTAL MICROBALANCE

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Abstract—Specific adsorption of biotinylated microbubbles to streptavidin was evaluated by measuring the resonant frequency of an AT-cut quartz crystal microbalance (QCM). Streptavidin was fixed *via* self-assembled monolayers coated onto the QCM electrode. The resonant frequency of the QCM decreased as a result of specific adsorption of the biotinylated microbubbles, compared with the results for microbubbles containing no biotin. Additionally, there was significant evidence indicating that the frequency shift was caused by the internal gas of the microbubble, as well as the mass of the outer-shell material surrounding the gas. These results suggest that the QCM measurement system can be used effectively to evaluate the specific adsorption of targeted microbubbles. (E-mail: buj3076@gmail.com or takashige.nnn@gmail.com) © **2014 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Biotin-avidin bonds, Lipid shell, Quartz crystal microbalance, Resonant frequency, Targeting, Ultrasound contrast agent.**

INTRODUCTION

Recently, ultrasound diagnostic imaging has attracted significant attention because of its tolerability, low cost, portability and applicability to real-time imaging. Lipid-based microbubbles act as contrast agents in ultrasound imaging and have potential therapeutic applications (Unger et al. 2004). For example, drugs can be incorporated into or onto microbubbles for use as drug carriers in a drug delivery system (Hernot et al. 2008). Genetic material, in particular, must be delivered to targeted locations and integrated into the target cells of a patient. Because it is still difficult to deliver genetic material into cells, delivery techniques, for example, sonoporation, have been extensively studied (Bekeredjian et al. 2005; Li et al. 2003). In contrast, molecular imaging with ultrasound relies on microbubbles' selective adherence to ligand-specific targets. Attaching ligands to the surface of microbubbles allows the microbubbles to be targeted to specific molecular markers expressed at tissue sites. The greater the concentration of microbubbles, the higher the ultrasound signal observed, permitting easy detection of the targeted site (Lindner et al. 2001).

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To achieve effective delivery of microbubbles to the targeted site, an understanding of the binding interaction of microbubbles is needed. Based on the reaction kinetics and binding strength between microbubbles and the targeting site, such a system, ideally, could describe the bubble size and oscillating motion of the microbubbles during ultrasound irradiation. The specific targeting of microbubbles has been evaluated using piezoelectric methods and surface plasmon resonance (SPR) (Cho et al. 2006; Joseph et al. 2005).

Surface plasmon resonance is a surface-sensitive non-linear optic method that has been widely used to detect biological interactions with high resolution. Using the coupling between evanescent waves and surface plasmons, this technique detects changes in the refractive index close to the sensor surface; changes in the index of refraction are proportional to changes in mass. Therefore, SPR quickly provides valuable information, such as the rate and extent of adsorption or degradation, without the need for ligand labeling. However, information other than changes in mass (*e.g.,* density, viscosity and acoustic-coupling phenomena) cannot be acquired using this method. Additionally, interactions several hundred nanometers from the surface cannot be detected, because the intensity of the plasmon signal decreases exponentially as depth into the sample medium increases. Hence,

SPR detects only specific ligands on the surface of microbubbles. Because the diameter of the microbubble is larger than the penetration depth of the evanescent wave, SPR cannot provide information on the effect of internal gas inside the targeted microbubbles.

Piezoelectric devices, such as the quartz crystal microbalance (QCM) and surface acoustic wave (SAW) sensor, can also be used to determine the targeting ability of microbubbles. QCM techniques are widely used to detect biomolecules in aqueous solutions. The QCM is known to be quite sensitive not only for the gas phase, but also for the aqueous phase. It is well known that the resonant frequency of the QCM decreases depending on the increase in mass on the QCM electrode. This phenomenon is referred to as the "mass loading effect" in this article. Thus, the QCM system is capable of detecting substances at the nanogram level, allowing its use as a DNA sensor (Okahata et al. 2000) and enzyme sensor (Anzai et al. 1997). In addition to mass loading effects, other characteristics, such as the viscosity and elasticity of layers adhering to the QCM surface, can be evaluated because these factors influence the resonance characteristics of the QCM. In the measurement of targeted microbubbles, those bound to the QCM surface may undergo translational motion in a direction parallel to the QCM surface. The drag force, which acts on the translating bubbles, may generate shear stress as the reaction force on the surface *via* the binding and changes the resonance characteristics of the QCM. The magnitude of the drag force depends on the amount of internal gas (*i.e.,* bubble size) (Magnaudet and Legendre 1998). Under this hypothesis, the total shear stress on a QCM depends on the size distribution and the concentration of the microbubbles attached to the surface. In other words, this information of the microbubbles may be obtained from the degree of shift in resonant frequency.

Joseph et al. (2005) found that targeted microbubbles could be detected with a SAW sensor. When SAWs propagate along the surface of a piezoelectric crystal, their energy is concentrated on the surface. Hence, the propagation characteristics of SAWs depend on the adjacent medium. In SAW sensors, frequency changes or phase shifts are measured to determine the physical and chemical properties of the medium, as well as the QCM. Therefore, it may be possible to detect the internal gas in microbubbles with a SAW sensor; however, the mechanism was not discussed in Joseph et al. (2005).

The purpose of this study was to evaluate specific targeting of microbubbles based on measurement of the resonant frequency shift using the QCM. We measured the specific interaction between biotinylated microbubbles and streptavidin coating a QCM electrode. The primary aim of this article was to illustrate that specific adsorption of microbubbles could be measured with the QCM system. Streptavidin-biotin binding has little meaning in clinical applications and is considered in more academic applications. However, it is suitable for achieving our purpose because it has high absorptivity compared with, for example, antigen-antibody adsorption. Many bubbles could be strongly adsorbed on the QCM surface *via* streptavidin-biotin binding. This effect could induce a large shift in QCM resonant frequency. As a secondary aim, we sought to determine whether a mechanism differed from the mass loading effect underlies the observed shift in resonant frequency. In the practical use of targeted microbubbles, there are many impurities (many types of proteins and lipids) around targeted sites in the human body. We must investigate the targeting ability of microbubbles in such a situation. If microbubbles cause the frequency shift in QCM by a mechanism differed from the mass loading effect, its effect should help us to measure the targeting ability of microbubbles separate from the other materials. The mechanism underlying the observed frequency shift was discussed, with a focus on the effect of the internal gas.

METHODS

QCM and experimental systems

AT-cut 5-MHz quartz crystals (QA-A5 M-AU(M) (SEP)) were supplied by Seiko EG&G Princeton Applied Research (Princeton, NJ, USA). The quartz surface was coated on both sides by gold electrodes. A lead wire was connected to both electrodes by tucking a QCM with it, as illustrated in Figure 1(a). The QCM was sandwiched by O-rings and fixed to the cell with screws. The subject liquid was deposited on one side of the QCM. Because the O-rings prevent the liquid from leaking, the opposite side is in the air. Various solutions could be dropped into the cell. For the AT-cut shear-mode QCM, the mass loaded on the QCM electrode decreased the resonant frequency (Sauerbrey et al. 1959). Thus, the QCM acts as a mass sensor. Using the QCM sensor, we attempted to evaluate the binding behavior of biotinylated microbubbles. In the experiment, we measured the reflection coefficient of the QCM sensor with a network analyzer (E5071B, Agilent Technologies, Santa Clara, CA, USA) (Fig. 1b). The peak frequency at which the reflection coefficient reached its minimum value was defined as the *resonant frequency*. We measured the third harmonic resonant frequency. The third harmonic frequency can be predicted on the basis of the relationship between the resonant frequency and the thickness of the QCM. Frequency characteristics of the reflection coefficient S_{11} in the vicinity of the predicted third harmonic were measured, as illustrated in Figure 2. The data were fitted using a quadratic curve. The frequency at which