Ultrasonics 63 (2015) 102–110

Contents lists available at ScienceDirect

Ultrasonics

journal homepage: www.elsevier.com/locate/ultras

Numerical analysis of acoustic impedance microscope utilizing acoustic lens transducer to examine cultured cells

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ARTICLE INFO

Article history:
Received 24 January 2015
Received in revised form 11 May 2015
Accepted 20 June 2015
Available online 2 July 2015

Keywords:
Ultrasound
Acoustic impedance
Glial
Glioma
Cytochalasin

ABSTRACT

A new technique is proposed for non-contact quantitative cell observation using focused ultrasonic waves. This technique interprets acoustic reflection intensity into the characteristic acoustic impedance of the biological cell. The cells are cultured on a plastic film substrate. A focused acoustic beam is transmitted through the substrate to its interface with the cell. A two-dimensional (2-D) reflection intensity profile is obtained by scanning the focal point along the interface. A reference substance is observed under the same conditions. These two reflections are compared and interpreted into the characteristic acoustic impedance of the cell based on a calibration curve that was created prior to the observation. To create the calibration curve, a numerical analysis of the sound field is performed using Fourier Transforms and is verified using several saline solutions. Because the cells are suspended by two plastic films, no contamination is introduced during the observation. In a practical observation, a sapphire lens transducer with a center frequency of 300 MHz was employed using ZnO thin film. The objects studied were co-cultured rat-derived glial (astrocyte) cells and glioma cells. The result was the clear observation of the internal structure of the cells. The acoustic impedance of the cells was spreading between 1.62 and 1.72 MNs/m^3. Cytoskeleton was indicated by high acoustic impedance. The introduction of cytochalasin-B led to a significant reduction in the acoustic impedance of the glioma cells; its effect on the glial cells was less significant. It is believed that this non-contact observation method will be useful for continuous cell inspections.

1. Introduction

Several methods are available for examinations of biological and medical specimens, such as X-rays, electromagnetic waves, and optical and acoustical microscopes. For microscopic images, optical rays have short wavelengths that provide very high resolution images. However, because it is not easy to obtain good image contrast, staining is needed. The staining process adds a contaminant to the target. In addition, it requires several hours to perform the staining process. Finally, heavy staining has the potential to alter the biological function of the target or even damage it, causing loss of the targets information.

On the other hand, scanning acoustic microscopy (SAM) is an advantageous method of non-destructive analysis with which the user can measure a samples properties without touching or damaging it. Because the staining and slicing processes are avoided, the time required for observation is very short. Furthermore, this method has the benefit that it can provide a quantitative evaluation of viscous-elastic or acoustic properties of the target [2–6].

Several researchers have used SAM for cell observation [1–5,7]. Vector contrast SAM was developed for imaging living stem cells. Signal processing, autofocusing and image processing are required for creating time-lapse motion pictures of mesenchymal stem cells [8]. Zang et al. utilized an atomic-force acoustic microscope to study the surfaces, intracellular structure and elasticity of Vascular Smooth Muscle Cells [9]. They mainly placed SAM instrumentation around the cell in the top to bottom order of transducer, coupler, cell and substrate. With this arrangement, they could measure the cell surface, its thickness or the speed of sound in the cell because they had access to the cells internal structure.

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http://dx.doi.org/10.1016/j.ultras.2015.06.016
0041-624X/

However, because the transducer, cell and coupling medium were integrated, it was possible to introduce a contaminant.

Kobayashi et al. reported successfully investigating the acoustic impedance of biological tissue [6]. They prevented target contamination during observation. Vertical incidence analysis was used to estimate the acoustic reflection intensity from a small angle of a focused transducer. In our last studies [10,11], a significant focus transducer was employed with frequencies in the 30–100 MHz range. Oblique incidence was taken into account, instead of only vertical incidence analysis. The soft tissue of a rat brain was successfully interpreted with acoustic intensity, which was subsequently converted into acoustic impedance utilizing a calibration curve. These studies provided two advantages: quantitative analysis and freedom from contaminants during observation.

We have used the previous study [11] for a smaller target (*i.e.*, cell structure instead of biological tissue). However, for viewing a cell, the image quality was reduced because the spatial resolution was too low. As the target shrinks, higher spatial resolution must be used to obtain a high-quality image. Hence, higher acoustic frequencies must be applied [12–14].

In this paper, we perform quantitative and contaminant-free measurements of cultured cells. We propose a calibration curve as a new method to estimate the acoustic impedance of a cultured cell. A transducer lens with a small radius of curvature is employed. To establish a calibration curve, the lens curvature shape, oblique incidence, frequency dependence and medium properties are considered. To treat multilayered media, we refer to Tittmann et al. [15] and the previous works [11]. Numerical calculations with Fourier Transforms are used to obtain acoustic intensities, and the resulting calibration curves are presented. Cell measurements and observations of the effects of drugs utilizing this system are also exhibited.

2. Material and methods

2.1. Diagram of system

Fig. 1 show a diagram of the SAM used in this research. Glial or glioma cells were used as targets. They were injected into a culture dish and preserved in an incubator before observations. The three major components are described in this chapter: the culture dish and culture method, the transducer, and the observation system.

2.1.1. Culture dish and culture method

An OptiCell Cell Culture System (Thermo Scientific Inc. MA, USA) was chosen to cultivate the cell. It had two polystyrene films with thicknesses of 80 m and a 2 mm gap. The polystyrene film is also used as a substrate during the measurement. Glial cells and glioma cells were cultured in the modified astrocyte-deficient medium [36]. Briefly, glial cells were prepared from neonatal VGAT-venus rat cerebellum, and cultured in 10% Calf-serum containing Hanks MEM with 4 g/L glucose and 0.02 g/L kanamycin. After one week incubation, purified and fluorescent glial cells were mixed with rat C6 glioma (DS Pharma Biomedical Co. Ltd. Osaka, Japan), incubated for 4 days before being observed.

2.1.2. Transducer

A transducer of ZnO piezoelectric material (Honda Electric, UPF-5A type) was used for both transmitting and receiving the acoustic wave pulse. The transducer was attached with a sapphire lens of (assumed uniform) half curvature of 60°, 0.41 mm aperture diameter and 0.25 mm radius. Pure water (speed of sound = 1480 m/s, density = 1000 kg/m^3) was chosen to couple the substrate and the transducer as shown in Fig. 1.

2.1.3. Flow work of system

A set of programs constructed under LabVIEW was run in a Personal Computer (PC). The PC could send two commands through a General Purpose Interface Bus (GPIB). The first command was a motion control that was sent to the stage controller (SIGMA KOKI, SHOT-204MS), which relayed the command to two stepper motors (SIGMA KOKI, serial number 1101585) for x–y scanning. The size of x and y axis steps was adjustable. The second command was sent to a MultiFunction Synthesizer (WaveFactory, model WF 1946B) to create a short pulse. Short pulses were cyclically generated (100 kHz) from the Synthesizer and sent to the transducer. The transducer converted the electrical pulses into ultrasound waves, and transmitted them from the bottom. The focal point was on the interface between the substrate and the cells. Adjusting the focal point was not easy; it was found by moving the transducer up and down until the maximum reflection intensity was received by the transducer. The ultrasonic beam reflected from the local area of the target with a frequency spread from 200 to 400 MHz (as shown in Fig. 8) was subsequently received by the same transducer, sent to the PC through an Agilent Acqiris U1065A (model DC222), and interpreted as acoustic intensity. By scanning the target, a 2-D image of reflection intensity was obtained.

2.2. Sound fields analysis

Fig. 2 illustrates the interpretation of reflection intensity as acoustic impedance. Reflection from the target is compared with that from the reference material. In the previous paper [6] it was assumed that the incidence was perpendicular to the substrate. This assumption might be appropriate when the angle of focus is small. However, in this study, the half angle of focus was as much as 60° to retain a high spatial resolution. Therefore, we performed sound field analysis to accurately calculate the relationship between reflection intensity and acoustic impedance.

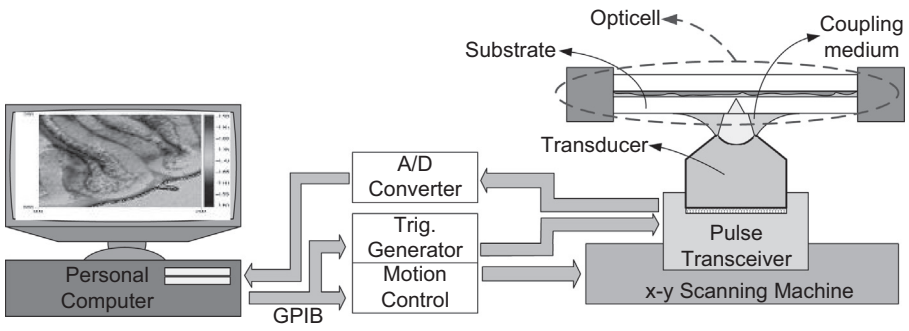

Fig. 1. Schematic diagram of the SAM [14].