Ultrasound Intensity Microscopy for Imaging of Living Cells

Yoshihiro Hagihara1, Eiichi Chimoto1, Akira Ando1, Yoshifumi Saijo2, Eiji Itoi1

1Orthopaedic Surgery, Tohoku University Graduate School of Medicine, Sendai, Japan; 2Institute of Development, Aging and Cancer, Tohoku University, Sendai, Japan

hagi@mail.tains.tohoku.ac.jp

Introduction: Acoustic microscopy for medicine and biology has been developed for more than twenty years at Tohoku University (1-3). Application of acoustic microscopy in medicine and biology has three major features and objectives. First, it is useful for intra-operative pathological examination because staining is not required. Second, it provides basic acoustic properties to assess the origin of lower frequency ultrasonic images. Third, it provides information on biomechanical properties at a microscopic level because ultrasound has close correlation with mechanical properties of the tissues. In the present study, ultrasound intensity microscopy was developed in order to visualize living cells. It realized non-contact high resolution imaging of cultured synovial cells from a rat.

Materials and Methods: Ultrasound intensity microscopy: An electric impulse was generated by a high speed switching semiconductor. The electric pulse was input to a transducer with sapphire rod as an acoustic lens and with the central frequency of 300 MHz. The reflections from the tissue was received by the transducer and were introduced into a Windows-based PC (Pentium D, 3.0 GHz, 2GB RAM, 250GB HDD) via a digital oscilloscope (Tektronix TDS7154B, Beaverton, USA). The frequency range was 1 GHz, and the sampling rate was 20 GS/s. Four values of the time taken for a pulse response at the same point were averaged in order to reduce random noise.

The transducer was mounted on an X-Y stage with a microcomputer board that was driven by the PC through RS232C. The Both X-scan and Y-scan were driven by linear servo motors.

The ultrasound propagates through the thin specimen such as cultured cells and reflects at the interface between the specimen and substrate. Two-dimensional distributions of the ultrasonic intensity was visualized with 200 by 200 pixels (Figure 1).

Figure 1: Ultrasound intensity microscopy. A, Block diagram of the ultrasound intensity microscopy. B, Schematic illustration of measurement.

Tissue preparation: The synovial membrane was obtained from non-operated male rats weighing from 380 to 400 g through medial para-patellar incision. The tissue was diluted and loosened 0.15% DispaseII (Boehringer, Mannheim) in DMEM for 2 hours at 37°C. Then centrifuged at 400 g for 5 min and discard the supernatant. The cells were plated in 75 mm² dish (Falcon) with Dulbecco’s modified Eagle’s medium (DMEM, GibCo Laboratories) containing 10% fetal bovine serum (SIGMA Chemical Co.) at 37°C in a CO2 incubator. To determine changes of intensity, the cells were treated with 1 ng/ml of human recombinant TGF-β1 (R&D Systems, Inc.) for 1 and 3 days after reaching confluent. The non-treated cells was harvested at 3 days after reaching confluent and defined as control. Randomized four points at each dish were measured and averaged data was defined as the representative value of each dish. The cells used for experiments were at the second passage.

Statistical analysis among groups was performed using the Kruskal-Wallis test with Bonferroni/Dunn post-hoc multiple comparisons. Data were expressed as mean ± standard deviation. A value of P < 0.05 was accepted as statistically significant.

Results: Ultrasound intensity microscopy can clearly visualize synovial cells. The high intensity area at the central part of the cell corresponded to the nucleus and the high intensity area at the peripheral zone corresponded to the cytoskeleton mainly consisted of actin filaments. The intensity at the actin zone was increased after stimulation with TGF-β1 (Figure 2).

Discussion: Ultrasound intensity microscopy realized high precision imaging of synovial cells and clearly visualized changes of ultrasound intensity after stimulation with TGF-β1. TGF-β is also a crucial regulator of ECM deposition, as it controls the expression of components of extra-cellular matrix, which includes type I collagen, type III collagen, and fibronectin in fibroblasts (4,5). The increased intensity after stimulation might be due to a deposition of extra-cellular matrix in the cells. Higher frequency transducer of the ultrasound intensity microscopy can obtain higher resolution images and sound speed data would clearly show quantitative biomechanical properties of the cells.