ACOUSTIC BACKSCATTER CHARACTERIZATION OF IN-VITRO FETAL LUNGS: DETERMINATION OF ALVEOLAR SACS SIZE

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INTRODUCTION

One of the major causes of newborn mortality is pulmonary immaturity. Fetal lung maturation is determined by biochemical analysis of the amniotic fluid phospholipids. This is an invasive procedure that is potentially harmful to the mother and fetus. Anatomical changes in the lung co-occur with biochemical changes [1]. The developing alveolus has a central cavity containing fluid. During maturation, as the wall of the alveolus becomes thinner (cf. Figure 1), the ratio of the volume of the central cavity to the total volume of the alveolus increases resulting in an increase in the percent fluid content per alveolus [2]. B-mode acoustic scattering techniques have been utilized to determine lung tissue structural properties [2][3]. Scattering from the fetal lung as a function of maturity has been attempted by analysing qualitatively and semi-quantitatively B-mode images of commercial US equipment. Although these scattering techniques have provided encouraging results with regard to assessing lung maturation, they remain highly dependent on the equipment used, and are subjective to the evaluation of the sonographer.

Therefore, to advance our knowledge of fetal lung development, there is a need to 1) seperate the acoustic scattering properties of fetal lung tissue from the properties of the measuring equipment using standardized tissue characterization methods 2) verify the validity of these methods to characterize the lung tissue by using standard phantoms, and 3) apply these methods to characterize fetal lung tissue, i.e., correlate acoustic measurement with tissue properties using quantitative techniques. These issues can be addressed using A-mode US which provides the means for quantitative, equipment independent, signal analysis of raw radio frequency (RF) data to yield a measure of acoustic scattering over a wide frequency range and with sufficient linearity.

The aim of this research is to examine the potential of A-mode US to accurately determine the size of alveolar sacs which is fundamental to the eventual determination of fetal lung development.

METHODS

SIZE ESTIMATION

The backscatter power spectrum of a signal reflected from a volume containing small randomly suspended scatterers was used to estimate average size. The rationale for the estimation of scatterer size from a tissue phantom containing small scatterers has been described elsewhere [4].

INSTRUMENTATION

The basic instrumentation is diagrammed in Figure 2. It consisted of a transmitter and a receiver (6.2 MHz, 12.7 mm diameter spherically focused at 50.8 mm) both driven by a Matec SR9000 pulser/receiver circuit. The received analog signal was digitized (Tektronix) and analyzed using a specially developed software that permitted setting the sampling frequency, and gating only a segment of interest in the signal.

CALIBRATION

For a valid tissue characterization experiment it is necessary to use a known tissue medium for instrument calibration, and henceforth, use this calibrated equipment to characterize the unknown lung tissue. A Tissue mimicking (TM) material (prepared at the Department of Medical Phyics and Engineering, University of Wisconsin, Madison) containing 4 mg/cm^3 microspherical glass scatterers of 45-53 μm in diameter with equal concentrations and spread homogeniously throughout the material was used for calibration. The mean spherical scatter diameter across the frequency range 2-8 MHz was equal to 43 ± 5 μm. Assuming the TM contains spheres of a mean diameter equal to 49 μm (average diameter of 45 and 53 μm spheres), the correlation coefficient between the estimated spherical scatterer diameter and the average diameter contained in the TM was equal to 0.97 (P value < 0.00024).

PROCEDURE

7 preterm lamb lungs (gestational age 134-141 days; mean age = 137 days, standard deviation = 3 days) obtained from pregnant ewes, were inflated (pressure = 18 ± 2 mmHg) and fixed with 10% formaldehyde. Samples representing only alveolar regions were cored from the upper, middle, and lower lobes of each lung for scattering measurements. 30 independent signals from each lung were obtained by translating the transducer perpendicular to beam direction and were analyzed in 3 μs time gates located in the focal zone of the insonifying transducer. To remove system effects on the reflected signals, echo signals were normalized with a plane perfect reflector. The same lung samples were used for histological analysis to verify the accuracy of the size estimation technique. 10 light microscopic images (256 256 pixels, 1.538 μm per horizontal pixel, 1.274 μm per vertical pixel) were digitized from each lobe in such a way to include only alveolar structures. Larger structures were excluded in order to measure only the mean alveolar sac surface area. Based on Weibel [5], software was developed to compute the mean alveolar sac surface area by dividing the total alveolar surface area over the total number of alveolar sacs inside the image sample.

RESULTS

Scatterer dimensions clustered in three size ranges; 1) a small size range, 20-150 μm, 2) a medium range, 150-250 μm, and 3) a large range 250-400 μm. The large and medium scatter sizes are believed to arise from bronchioli, respiratory and terminal bronchioli. Results from morphometric analysis were compared to the *small size* scattering range obtained from acoustic scattering. The effective scatterer diameter acoustically estimated and averaged over the 7 lambs data was 81.6 ±1.5 μm when only scatterers at high frequencies (small size scatterers) were considered. The mean alveolar sac size, histologically determined, was 87.0 ± 3.0 μm.

DISCUSSION AND CONCLUSION

The close values between the mean effective scatterer diameter and the mean alveolar sac size suggested that the primary sources of scattering were the collagen rich septal walls of the alveoli which makeup the alveolar sacs. Therefore, A-mode US can provide accurate structural information about the fetal lung. Further research will examine the sensitivity of A-mode US to structural changes within different regions of the lung and throughout gestation in-vitro and in-vivo.

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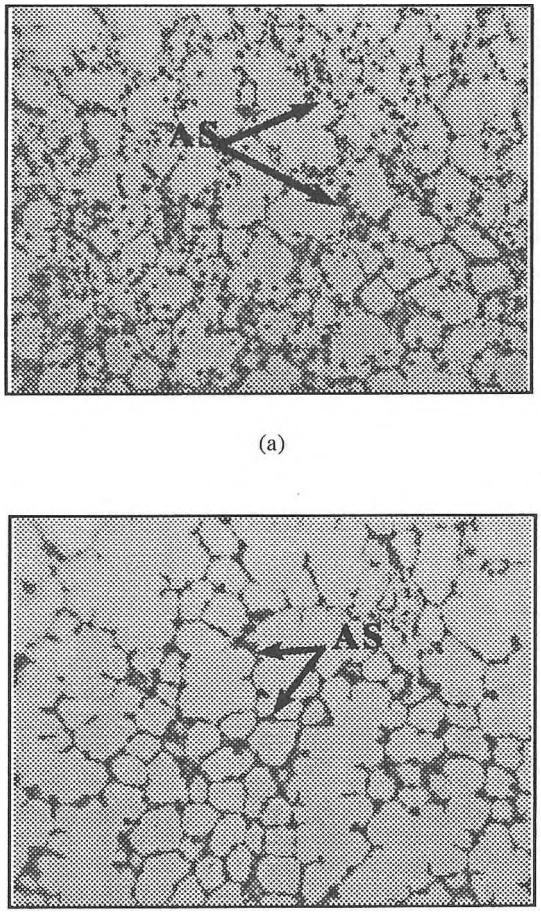

(a)

(b)

Figure 1. Alveolar Sac (AS) septal walls appear to be thicker at 134 days of gestation (a) than those at 141 days of gestation (b) in the sheep fetal lamb.

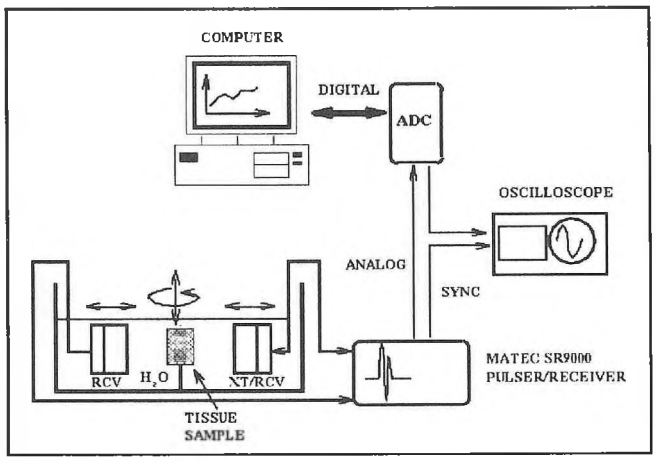

Figure 2. Basic Instrumentation.